Nearshore Ecology (NSE) of Grand Canyon Fish

Final Report

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List of abbreviations
Fish species – Common name
BBH – Brown bullhead
BKC – Black crappie
BNT – Brown trout
CCF – Channel catfish
CRP – Common carp
FHM – Fathead minnow
FMS – Flannelmouth sucker
GSF – Green sunfish
HBC – Humpback chub
PKF – Plains killifish
RBT – Rainbow trout
RSH – Red shiner
SPD – Speckled dace
STB – Striped bass
SUC – Unidentified sucker

Places
CR – Colorado River
LCR – Little Colorado River

Gear
EF – Boat electrofishing
HN – Hoopnets

Other
NSE – Nearshore Ecology Project
UF – University of Florida
GCMRC – United States Geologic Survey Grand Canyon Monitoring and Research Center
PIT – Passive Integrate Transponder, a tag type that assigns a uniquely identifiable identification to each fish
VIE – Visible Implant Elastomer, a tag type that provides a batch mark that identifies the trip, gear, and site where a fish was collected
HSU – Habitat Sub-unit, spatially referenced 50-m sections of shoreline that were delineated and numbered throughout the three NSE study reaches.

Note: All information presented is either published, submitted for publication, or being prepared for submission for publication in peer-reviewed journals. For published articles we have provided a reprint of the article. Because different parts of the project are being submitted to different journals, slight variations in page layout, style, and references are used throughout this report which reflects the style of the target journal. We recommend referencing peer-reviewed articles as the final products from this research project.
Executive Summary Nearshore Ecology Project

Introduction- Management actions in aquatic ecosystems are frequently directed at restoring or improving specific habitats to improve fish growth and survival. In the Grand Canyon reach of the Colorado River experimental flow operations as part of the Glen Canyon Dam Adaptive Management Program impact the creation of habitat types (i.e., sandbars and associated backwaters), water temperatures, or flow fluctuations and magnitude which are critical for juvenile native fish conservation. One of the objectives of a recent experiment evaluating steady flow operations from Glen Canyon Dam was to understand how river discharge, through its interaction with physical habitat structure, influences the growth, survival rates, and habitat use of juvenile native fish in the Colorado River in Grand Canyon. The key fish species of interest was humpback chub *Gila cypha*, currently listed as “Endangered” under the US Endangered Species Act. Population recovery of humpback chub is of keen interest to numerous federal, state, and tribal entities. The “Nearshore Ecology” (NSE) project was designed to address key fundamental research questions to improve our knowledge of humpback chub population ecology.

(1) Do steadier flows during late summer and early fall increase survival, abundance, and/or growth rates of juvenile native fish?

(2) Do juvenile humpback chub select specific habitat types and if so, does this selection change under different river flow regimes?

(3) What is the provenance of juvenile humpback chub in the mainstem Colorado River and is there evidence for mainstem spawning and rearing?

Background- Glen Canyon Dam is operated primarily as a load-following hydropower facility, increasing and decreasing dam discharge to match power demand. This periodic “flushing” of the river reduces the residence time of water as well as the availability of certain shoreline habitat types. In contrast, steady flows can increase the retention time of water in littoral areas such as backwaters and low-angle shorelines, and if discharge volume and ambient temperature are appropriate, can locally increase water temperatures. The NSE project
evaluated experimental steady flows that occurred from 1 September-31 October in each year 2009-2011. This study took place between river km 102-106 just downstream of the confluence of the mainstem Colorado and Little Colorado rivers where most prior research on humpback chub in Grand Canyon has occurred. The timing and magnitude of the steady flow experiment was developed by resource managers independent of the NSE team. Experimental flow regimes were about 10% of the unregulated (pre-Glen Canyon Dam) annual fluctuations.

Prior to the NSE project, our understanding of juvenile humpback chub ecology in the mainstem Colorado River was deficient compared with our knowledge of adult humpback chub primarily because of limited sampling of mainstem habitat for juvenile life stages. The NSE project developed a sampling and analytical framework to directly assess juvenile humpback chub survival, abundance, individual growth, and habitat use. These analyses used spatially referenced mark-recapture experiments with multiple gear types and determined provenance (birth river) through otolith microchemistry. This direct assessment of key vital rates complements indirect approaches used to estimate survival through population modeling efforts. For example, age-structured-mark-recapture (ASMR, Coggins et al. 2006) reconstructs juvenile abundance and survival through time from adult population numbers (estimated from mark-recapture) and assumes survival relationships based on life-history characteristics and growth rates. In contrast, the NSE project directly estimates juvenile fish population metrics in terms of abundance, survival, growth, or habitat use, which is useful for rapidly assessing how juvenile humpback chub respond to management actions such as experimental flows.

Results and Discussion- The NSE project found that annual apparent survival of juvenile humpback chub (size at tagging < 100-mm total length, TL) did not differ significantly between the extant fluctuating flows and the experimental steady flow treatments (Finch et al. in-review A). The NSE project also documented that juvenile humpback chub were able to survive and rear in the mainstem Colorado River even at small sizes of 40-100 mm TL (Finch et al. in-review B). A somewhat surprising finding was that growth in juvenile humpback chub declined during these short-term steady flows versus fluctuating flows even though water temperatures were generally similar (Finch et al. in-review B). Reasons for this counterintuitive growth response...
are not known, but Finch (et al. *in-review B*) hypothesizes that food availability in the drift (primarily aquatic insects) is higher in fluctuating flows than in steady flows.

In Grand Canyon the creation, maintenance, and persistence of specific habitat types are considered critical for the persistence and recovery of native fish populations, including humpback chub. Backwaters are thought to be more similar to the Colorado River ecosystem prior to river modification because they are generally warmer and may be less influenced by river stage and dam operations than other mainstem habitat types. The NSE study compared abundance, density and habitat selection patterns between shoreline habitats (cliff, talus, debris fan, sand, backwater) and found that abundance of juvenile humpback chub was consistently highest in talus habitats and lowest in backwater habitats (Dodrill et al., *in-review*). Juvenile humpback chub showed positive selection for backwater habitats, but the spatial extent of backwater habitats in the NSE study reach was small compared to other habitat types. Additionally, ultrasonic telemetry of larger juvenile humpback chub (about 180-190-mm TL) found that habitat selection and daily movements did not change between fluctuating conditions and the steady flow experiment (Gerig et al., *in-review*). This suggests that, at least in this reach of the Colorado River, management actions directed at manipulating backwater habitat type will affect only a small proportion the habitats, and the population, of humpback chub. The NSE study reach is in a section of Grand Canyon with steep bank angle, thus the available habitat in this reach is relatively unaffected by changes in river stage associated with the range of flows observed from 2009-2011. Future work could assess whether juvenile humpback chub are similarly robust to changes in a river reach where available habitats are more flow sensitive.

We successfully developed a framework to address humpback chub growth and provenance through the analysis of humpback chub otoliths (ear-stones). Otoliths form part of the hearing and balance system in fishes and grow incrementally as fish grow. Made of calcium carbonate, otoliths absorb trace elements and isotopes from the environment. If these differ amongst environments, analysis of otolith chemistry can shed light on provenance and lifetime habitat use of fishes. Direct querying of this sort permits deeper insight into population ecology and habitat use, because individual fish life histories are tracked retrospectively. Data from
tagging programs, on the other hand, are limited to the locations in which fish are captured and recaptured.

Using information on water and humpback chub otolith chemistry we asked:

(1) Do different parts of the mainstem Colorado River system in Grand Canyon, including its tributaries, differ in terms of dissolved water chemistry?

(2) In particular, are there trace elemental and/or isotopic markers that can distinguish the Little Colorado River tributary from the mainstem? If so, then

(3) At what age and size do juvenile humpback chub emigrate from the Little Colorado to the mainstem Colorado River?

(4) Is humpback chub recruitment to the mainstem Colorado River population dependent on reaching a certain size before emigration?

(5) Are any humpback chub spawned in the Colorado mainstem and if so do they survive?

We developed a geochemical atlas of the Grand Canyon reach of the Colorado River, analyzing a suite of trace elements and stable isotopic ratios. Our sampling was limited primarily to summer and fall months in 2009 – 2012. We assayed otoliths of juvenile humpback chub as well as any available adult chub that had been collected as incidental mortalities.

We found that the ratio of a few trace elements to calcium – primarily strontium, barium, and selenium, along with the stable isotopic ratios of carbon, oxygen, and hydrogen/deuterium could, as an ensemble, serve to discriminate between the mainstem and the Little Colorado River, which was the focus of the study (Hayden et al., 2012; Limburg et al., in review). Carbon stable isotopic ratios ($\delta^{13}C$) were particularly good discriminators because of the substantial fractionation during travertine formation within the Little Colorado River. We also found that the chemical signature of the mainstem was maintained throughout the entire reach of Grand Canyon, with little variation, such that this signature was readily distinguished from many of the tributaries.

We measured lapillar otoliths of humpback chub and derived a relationship between otolith size and fish total length. Using this relationship, we back-estimated the size, as well as
age (in days) when humpback chub egressed from the Little Colorado to the mainstem (Limburg et al., in-review). In addition to this evidence from changes in otolith chemistry we discovered changes in the otolith microstructure itself. Daily growth increments of otoliths reduced in width markedly upon fish entry into the mainstem due to the temperature differential which strongly affects otolith growth.

We determined a range of sizes and ages at egress amongst juvenile humpback chub captured in the mainstem. However, this range was reduced in adult chub, suggesting that successful recruitment (i.e. survival) is favored by remaining longer in the Little Colorado. We also found that fish captured in the Little Colorado tended to be larger on their first birthdays (determined retrospectively by otolith analysis) than fish in the mainstem. These fish also showed a bimodal distribution of size at age-1 suggesting differential growth within the birth cohort.

We analyzed a small number of juvenile humpback chub that had been captured many kilometers upstream of the Little Colorado confluence with the Colorado mainstem. These individuals turned out to be much older than comparably sized juveniles collected in the Little Colorado River. Chemical analysis of their otoliths suggested a different natal source; one source appears to be in the 30-Mile Spring reach and another may have been an unidentified spring or tributary (Hayden et al. 2012). Such small fish (20-25 mm) for their age (e.g., 70-80 days old) are unlikely to survive and recruit to adulthood in the mainstem Colorado River.

Conclusions- The NSE project developed a sampling and analytical framework to directly assess juvenile humpback chub population responses to management actions at smaller fish sizes than were previously possible. This framework is important, as the key outcome from many different types of management actions in the Colorado River is to improve survival of juvenile humpback chub, increasing overall abundance and accelerating the population to recovery. The NSE project also documented that small juvenile humpback chub can survive and rear in the mainstem Colorado River. This information is important because adult humpback chub numbers (age 4+) have increased over the past decade, possibly due to improved survival in the mainstem Colorado River (Coggins and Walters 2009).
We identified chemical markers that can distinguish fish use of Little Colorado River from mainstem use. Humpback chub in this reach of Grand Canyon originate overwhelmingly from the Little Colorado. Mainstem adult otoliths showed evidence that longer rearing in the Little Colorado promotes better growth and recruitment. The combination of otolith chemistry and growth increment analysis together produced a good natural marker that could be used as a tag to follow fish movements between the mainstem and Little Colorado River. Further work will be needed to extend this methodology to other humpback chub aggregations within Grand Canyon and possibly to other native fish species assessments.

The results of the NSE project suggest that juvenile humpback chub survival, growth, abundance, and habitat use are robust to the fall steady flows observed during 2009-2011. It is likely that more extreme flow treatments (e.g. higher or lower discharges, longer duration) are required before changes in these metrics would be observed. This research demonstrates the apparent flexibility of juvenile humpback chub in habitat selection regardless of fluctuating or steady river flows. Our development and application of methods to assess the growth, survival, and persistence of juvenile humpback chub in the mainstem Colorado River are key new additions to the body of knowledge available for managing the Colorado River and understanding how juvenile fish populations respond to hydropower operations in regulated rivers globally.

References


*Dodrill, M. J., B. S. Gerig, C. B Yackulic, W. E. Pine, C. Finch, and J. Korman. In-Review. Nearshore habitat use patterns of juvenile and small-bodied native fish in the Colorado River, Grand Canyon. Canadian Journal of Fisheries and Aquatic Sciences


*Available in this report
Introduction & Study motivation – The primary goal of this project was to understand how river flow, through its interaction with physical habitat structure, influences the survival rates of juvenile native and non-native fishes in the Colorado River in Grand Canyon. This was accomplished through a flow experiment from Glen Canyon Dam where water releases from the dam transitioned from fluctuating flows that followed hydropower demand to an approximately 60-day steady flow period before returning to fluctuating flows. Nine research questions related to this goal were identified in the original RFP (RFP pages 27-28); these questions had a hierarchical structure and varied in scope. Research addressed fundamental and process-oriented questions (e.g., do river flow conditions alter juvenile native fish survival rates?) as well as methodology (e.g., how to measure juvenile fish abundance, can small fish be marked?). Some research questions were quite specific (e.g., what is the Colorado River mainstem survival rate for humpback chub emigrating from the Little Colorado River during freshets?). A few questions require a longer time-frame of study than the length of this project (e.g., how do biotic and abiotic factors influence individual fish growth and survival by habitat type?) while others were quickly addressed within the project time-frame (e.g., what is the feasibility of marking small humpback chub?).

We followed three key fundamental research questions (RQ) throughout this project:

(RQ1) Do steadier flows during fall increase survival rates of juvenile native fish?

(RQ2) To what extent does physical habitat structure in conjunction with river flow patterns influence survival rate, habitat use, and abundance of juvenile native fish?

(RQ3) What is the provenance of juvenile humpback chub in the mainstem Colorado River and is there evidence for mainstem spawning and rearing?

We addressed these key research questions by developing a conceptual model for humpback chub *Gila cypha* (HBC) a species of significant research and management interest in the Grand Canyon (below). We framed these research questions around this conceptual model and then
tested different aspects of the model through our field work. We developed a methodological approach to assessing shifts in fish density by tracking habitat specific abundance and survival of native and non-native fish in response to nearshore habitat availability which were created and changed by the fall steady flow experiment. We then determined the source populations of juvenile native fish that populate nearshore habitats created by the steady flow experiment. Overall our project sheds new insight into juvenile fish ecology, with a focus on humpback chub. Our research findings create a better understanding of how flow and habitat management capabilities of the Glen Canyon Dam Adaptive Management Program (GCDAMP) can be used to cultivate and enhance survival of juvenile native fish and, with time, adult native fish populations in Grand Canyon.

**Conceptual Model** – The questions that motivated this project are part of a broader conceptual model of native and non-native fish population dynamics developed over the last 10-15 years in Grand Canyon. Such models have a long history of development in the Glen Canyon Dam Adaptive Management Program (e.g., Walters et al. 2000). We summarize below the essential elements from this model for juvenile humpback chub as they relate to this project:

**A. Humpback chub juveniles recruit to the mainstem humpback chub population from the Little Colorado River as very small juveniles during the spring. These small juveniles likely do not recruit to adulthood. Larger humpback chub juveniles recruit to the mainstem during late-summer and fall possibly related to monsoon-driven flood events in. These larger juveniles are the individuals most likely to survive to adulthood. Successful recruitment to adulthood from mainstem spawning, if it occurs, is likely low.**

**B. The quantity and quality of juvenile habitat in the mainstem is driven by variation in flow and temperature regimes and channel morphology. Habitat characteristics are determined by the monthly average discharge and hourly variation in discharge from Glen Canyon Dam, as well as sediment supply in the mainstem. The frequency and timing of flows from Glen Canyon Dam can be designed to create habitat believed to be important for native fish (e.g., backwaters).**
C. Survival rates of humpback chub juveniles in the mainstem depend on the quantity and quality of physical habitat, food availability, and the intensity of competition and predation from both native and non-native fishes.

D. Abundance of humpback chub juveniles will increase with improvements in the quality and quantity of habitat because survival rates will be higher. Over the long-term, greater juvenile production will increase the abundance of the adult population.

Summary information on the research site, experimental design, river conditions, and basic catch data from our field efforts from 2009-2011 is provided below. The following chapters are organized as stand-alone manuscripts that are either published, in-review, or in-preparation for submittal to peer-reviewed journals. We recommend referencing these critically evaluated manuscripts as the products resulting from this work.

2009-2011 Colorado River Research Site and Conditions – The Grand Canyon reach of the Colorado River is a roughly 400 km river section with boundaries on the western downstream end at Lake Mead and the eastern upstream end at Lake Powell (Figure 1), the first and second largest reservoirs in the United States, respectively. This river reach is contained within the borders of Grand Canyon National Park and is a UNESCO World Heritage Site, in addition to being listed as a federally protected region of cultural, geologic, and biological significance under the Grand Canyon Protection Act of 1992. Average discharge of the Colorado River through Grand Canyon for the past decade (2000-2010) was 351 m$^3$/sec as measured at the Phantom Ranch gauging station 145 km below Lee’s Ferry (rkm 145) and 171 km below Glen Canyon Dam. River flows in this river reach are primarily influenced by water releases from Glen Canyon Dam and inflows from the Little Colorado River tributary.

During our study typical fluctuating hydropower flows from Glen Canyon Dam occurred in July and August 2009 and 2010, while September and October samples from all years represented the fall steady flow experiments (Figures 2 and 3). Under fluctuating hydropower flows diel water releases occur to match electricity demand. During steady flow experiments water releases from Glen Canyon Dam were held constant at different rates each year. During
July and August 2011 Colorado River discharge from Glen Canyon Dam was high and steady as a result of required equalization flows between lakes Powell and Mead during a wet year in the upper Colorado River basin. During September and October of 2011 river flows were lower, but continued to be steadier than the preceding two months.

The Little Colorado River is the largest tributary of the Colorado River within Grand Canyon National Park and contains the Little Colorado River population of humpback chub. This population differs from other aggregations of humpback chub in Grand Canyon because only this one is known to complete all of its life stages. The Little Colorado River collects run-off from approximately 44,000 km$^2$ in northern and central Arizona with a mean annual discharge of 11.54 m$^3$/sec since 2004. Discharge from the Little Colorado River can influence river stage and turbidity in our study reach within the mainstem Colorado River. The hydrology of the Little Colorado River upper basin has been modified due to human settlement (see Figure 4 for a summary of Little Colorado River flows during the NSE study) but the river still retains seasonality in its discharge and temperature patterns.

Colorado River within Grand Canyon is stenothermic and cool due to the heat storage effect of Lake Powell and hypolimnetic water releases from Glen Canyon Dam (GCD). Annual GCD water release temperatures fluctuate around 2°C (from 8°C to 10°C from 1994-2002), with higher fluctuations and temperature maxima observed in recent years with fluctuations as high as 7°C (from 8°C to 15°C in 2011, Figure 5). One of the desired outcomes from the steady flow experiment was the warming of nearshore habitats due to steady water levels leading to water warming from ambient air temperatures. Nearshore water temperature responses to the steady flow experiment were negligible and reasons are assessed separately from this report (Ross and Grams 2013). Overall, mainstem water temperatures were warmer during 2009-2011 compared to historic post-Glen Canyon Dam water temperatures.

2009-2011 NSE Experimental Design – Field efforts in 2009-2011 included a total of four sampling trips each year, two trips before and two trips after the start of the experimental steady flow on September 1. Trip launch dates were mid-July, mid-August, early September and mid-October. Our sampling universe covered an area from Heart Island (just downstream of
the Little Colorado River confluence) to an area just upstream of Lava Chuar rapid (rmk 102.1 to rkm 104.7, Figure 1). Within this sampling universe we established three sampling sites (Sites 1, 2, and 3) of approximately equal length (about 1500-m) depending on hydrologic features of the river in that area. Each site was then subdivided further into individual spatially-referenced 50-m segments (“habitat sub-unit”, HSU). We used slow-speed boat electrofishing during night time to sample each HSU. Fish captured in each HSU were placed in a numbered bucket corresponding to the HSU to track catch spatially by HSU. All fish collected in each HSU were identified, measured following standard Grand Canyon fish sampling protocols (Persons et al. 2012) and given one of two tag types. We examined all native fish for Passive Integrate Transponder (PIT) tags. We tagged HBC greater than 100-mm TL and all other natives greater than 150-mm TL with a PIT tag. All native fish less than 100-mm TL and fathead minnows received a Visual Implant Elastomer (VIE) mark that identified gear used and site (1, 2, or 3) where the fish was captured (these marks are not unique to individual fish). The use of VIE marks was required because fish this size are simply too small to mark with PIT tags. We sampled each site over multiple nights and kept track of the cumulative numbers of captures and recaptures of fish (all species and tag types). We then used this information to estimate fish abundance for each site. Because different sampling gears have different sampling selectivities for a given fish species, fish size, or habitat, we used an additional technique to sample juvenile fish in Site 1 and Site 2. We employed standardized mini-hoopnets used by cooperating agencies for mainstem fish sampling (approximately 0.5-m in diameter, 1.0-m length, 6-mm mesh, and single 10-cm throat). In 2009 48 hoops in the upper 1.3 km of the sampling universe were used in July and, 60 hoops in Aug-Oct, all of which were fished 12 consecutive days for ~24 hours each. In 2010 and 2011 80 hoops over the entire 2.5 km sampling reach were used, also for 12 passes. All fish collected by hoopnets were processed similarly to the fish captured via electrofishing.

Water Chemistry and Isotope Sampling Overview – Water samples were collected from July – October 2009–2011, with special collections made in May 2009, June 2010, and September 2012. Acidified (1% HNO₃) samples were analyzed for trace elements with inductively coupled
plasma optical emission spectrometry (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS). Our limit of detection for most elements was in the low parts per billion. Following completion of quality control samples, results were only accepted if relative standard deviation (standard deviation / mean * 100) < 10%. Unacidified samples were analyzed for stable isotopic ratios of strontium, oxygen, hydrogen, and carbon. Strontium was analyzed in the Department of Earth Sciences, Syracuse University, using thermal ionization mass spectrometry (TIMS). Oxygen (delta$^{18}$O) and hydrogen:deuterium (H:D) ratios were analyzed by isotope ratio mass spectrometry at the Center for Stable Isotope Biogeochemistry at the University of California at Berkeley. Carbon (delta$^{13}$C) ratios were analyzed at the Stable Isotope Facility at the University of California at Davis.

Basic Fish Catch Data — From all four sampling trips in all sites, using both gears, we collected nearly 35,000 fish of 15 identifiable species and a group of unidentifiable suckers, mostly larvae (Table 1). Unidentified suckers were not retained due to concern that these might be humpback chub. The most abundant four species caught (by number) were native humpback chub, flannelmouth sucker, nonnative fathead minnows, and rainbow trout. We focus the remainder of this summary on catch information for humpback chub given the management interest for this species.

Size frequency analyses show that both gears captured a wide size range of humpback chub. Total catch of all sizes of humpback chub was higher in hoopnets than from electrofishing but electrofishing generally captured smaller fish than hoopnets (Figures 7-9). Overall, hoopnets were more effective at capturing total numbers of humpback chub, but our slow speed electrofishing technique appeared to be more effective at capturing the smallest size humpback chub. Closer examination of the density of humpback chub TL by sampling trip (July, August, September, October; Figures 10-12) show a range of interesting patterns with modal progression in size apparent during subsequent months in some years (e.g., 2011, Figure 12). In other years (e.g., 2009-2010 Figures 10 and 11) smaller humpback chub were captured in September and October that were not as readily apparent in the July and August samples.
To examine the spatial distribution of humpback chub catch, we created a plot of humpback chub catch by size class (delineated by tag type) on habitat sub-unit (HSU; Figures 13-18). The HSU represents the spatial grid cell of each electrofishing transect sample or hoopnet set. We structured this plot such that the HSUs for river right (sites 140-300) are found on the primary x-axis and the HSUs for river left (HSU 450-650) are found on the secondary x-axis. The catch in each of these grid cells (y-axis) then correspond to each x-axis such that catches close to zero for a given HSU are near the axis corresponding to that HSU (either primary or secondary x-axis) and non-zero catches are a greater distance away from the corresponding x-axis. Catches from electrofishing of humpback chub of all sizes by gear and sampling trip were widely distributed throughout each site. A similar pattern is apparent for humpback chub catches from hoopnet samples in sites 1 and 2 where hoopnets were used. The relationships between humpback chub habitat use, humpback chub density by habitat type, and abundance by habitat type are explored in detail in later chapters of this report.

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support through the project. The University of Florida College of Agricultural and Life Sciences provided graduate fellowships to B. Gerig and M. Dodrill to support their graduate degree programs at the University of Florida.
Table 1: Catch composition by fish species, sampling trip, and site site in the study reach of the Colorado River (rkm 102.1 to rkm 104.7) 2009-2011. Numbers of each fish species caught are shown; trips are identified by launch date (GC-year-month-day) 2009-2011.

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NSE Final Report Page 18
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Figure 1. Map of study area near confluence of Colorado and Little Colorado Rivers in Grand Canyon, northern Arizona, USA. Black box denotes the area containing the Little Colorado River aggregation of humpback chub.
Figure 2. Colorado River discharge August 22nd-September 10th, 2009 at the Lee’s Ferry gauge, 98 km upstream from NSE sampling universe. The period before September 1st is representative of normal hydropower operations, while the period after September 1st is representative of the steady flow experiment. Data from USGS instantaneous data archive. Available: http://ida.water.usgs.gov
Figure 3. NSE sampling calendar. Steady flow experiments occurred in September and October 2009-2010 and April-November 2011. Sampling periods are represented by the diagonally striped boxes. Discharge data available at 
http://waterdata.usgs.gov/az/nwis/rt
Figure 4. Daily discharge in the Little Colorado River near the confluence with the Colorado River over the three-year period of the Nearshore Ecology Study (2009-2011). Sampling periods are represented by the diagonally striped boxes. Data from USGS discharge gauge 09402300. Available: waterdata.usgs.gov (February 2012).
Figure 5. Colorado River water temperatures as measured at Lee’s Ferry, 26.4 km downstream from Glen Canyon Dam. The solid gray line is the mean temperature from 1994-2002 (repeated three times), while the dotted gray line is the mean temperature from 2003-2008 (repeated three times). The solid black line is the temperatures observed during each of the three years of the NSE study (not an average).
Figure 6. Map of sampling sites sampled during the Nearshore Ecology Project 2009-2011 in the mainstem Colorado River (Sites 1, 2, and 3) and also the standard USFWS sampling reaches in the Little Colorado River (Boulders, Coyote, Salt) that are sampled annually. Chute Falls the upstream migratory barrier for adult humpback chub is also shown in the Little Colorado River.
Figure 7. Length frequency (TL-mm) distributions for humpback chub collected during each sampling trip in 2009 (rows) by gear (each column) during the Nearshore Ecology Project.
Figure 8. Length frequency (TL-mm) distributions for humpback chub collected during each sampling trip in 2010 (rows) by gear (each column) during the Nearshore Ecology Project.
Figure 9. Length frequency (TL-mm) distributions for humpback chub collected during each sampling trip in 2011 (rows) by gear (each column) during the Nearshore Ecology Project.
Figure 10. Kernel density plot of humpback chub total length (TL-mm) collected as part of the Nearshore Ecology Project in the mainstem Colorado River using hoopnets and electrofishing. Each color represents a different sampling trip (July-October) for 2009.
Figure 11. Kernel density plot of humpback chub total length (TL-mm) collected as part of the Nearshore Ecology Project in the mainstem Colorado River using hoopnets and electrofishing. Each color represents a different sampling trip (July-October) for 2010.
Figure 12. Kernel density plot of humpback chub total length (TL-mm) collected as part of the Nearshore Ecology Project in the mainstem Colorado River using hoopnets and electrofishing. Each color represents a different sampling trip (July-October) for 2011.
Figure 13. Spatial distribution of humpback chub caught during each trip in 2009 (rows) by electrofishing. Circles indicate fish caught and marked with VIE (< 100-mm TL) and X indicate fish tagged with PIT tags (>100-mm TL). Habitat Unit (primary and secondary x-axis) represents spatially referenced 50-m shoreline sub-reaches. Primary x-axis represents river right HSU sites (facing downstream) and secondary x-axis represents river left HSU sites.
Figure 14. Spatial distribution of humpback chub caught during each trip in 2010 (rows) by electrofishing. Circles indicate fish caught and marked with VIE (< 100-mm TL) and X indicate fish tagged with PIT tags (>100-mm TL). Habitat Unit (primary and secondary x-axis) represents spatially referenced 50-m shoreline sub-reaches. Primary x-axis represents river right HSU sites (facing downstream) and secondary x-axis represents river left HSU sites.
Figure 15. Spatial distribution of humpback chub caught during each trip in 2011 (rows) by electrofishing. Circles indicate fish caught and marked with VIE (< 100-mm TL) and X indicate fish tagged with PIT tags (>100-mm TL). Habitat Unit (primary and secondary x-axis) represents spatially referenced 50-m shoreline sub-reaches. Primary x-axis represents river right HSU sites (facing downstream) and secondary x-axis represents river left HSU sites.
Figure 16. Spatial distribution of humpback chub caught during each trip in 2009 (rows) by hoopnets. Circles indicate fish caught and marked with VIE (< 100-mm TL) and X indicate fish tagged with PIT tags (>100-mm TL). Habitat Unit (primary and secondary x-axis) represents spatially referenced 50-m shoreline sub-reaches. Primary x-axis represents river right HSU sites (facing downstream) and secondary x-axis represents river left HSU sites.
Figure 17. Spatial distribution of humpback chub caught during each trip in 2010 (rows) by hoopnets. Circles indicate fish caught and marked with VIE (< 100-mm TL) and X indicate fish tagged with PIT tags (>100-mm TL). Habitat Unit (primary and secondary x-axis) represents spatially referenced 50-m shoreline sub-reaches. Primary x-axis represents river right HSU sites (facing downstream) and secondary x-axis represents river left HSU sites.
Figure 18. Spatial distribution of humpback chub caught during each trip in 2011 (rows) by hoopnets. Circles indicate fish caught and marked with VIE (< 100-mm TL) and X indicate fish tagged with PIT tags (>100-mm TL). Habitat Unit (primary and secondary x-axis) represents spatially referenced 50-m shoreline sub-reaches. Primary x-axis represents river right HSU sites (facing downstream) and secondary x-axis represents river left HSU sites.
Of Travertine and Time: Otolith Chemistry and Microstructure
Detect Provenance and Demography of Endangered Humpback Chub in Grand Canyon (USA)

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Abstract

We developed a geochemical atlas of the Colorado River in Grand Canyon and in its tributary, the Little Colorado River, and used it to identify provenance and habitat use by Federally Endangered humpback chub, *Gila cypha*. Carbon stable isotope ratios ($\delta^{13}$C) discriminate best between the two rivers, but fine scale analysis in otoliths requires rare, expensive instrumentation. We therefore correlated other tracers (Sr, Ba, and Se in ratio to Ca) to $\delta^{13}$C that are easier to quantify in otoliths with other microchemical techniques. Although the Little Colorado River’s water chemistry varies with major storm events, at base flow or near base flow (conditions occurring 84% of the time in our study) its chemistry differs sufficiently from the mainstem to discriminate one from the other. Additionally, when fish egress from the natal Little Colorado River to the mainstem, they encounter cold water which causes the otolith daily growth increments to decrease in size markedly. Combining otolith growth increment analysis and microchemistry permitted estimation of size and age at first egress; size at first birthday was also estimated. Emigrants < 1 year old averaged 51.2 ± 4.4 (SE) days and 35.5 ± 3.6 mm at egress; older fish that had recruited to the population averaged 100 ± 7.8 days old and 51.0 ± 2.2 mm at egress, suggesting that larger, older emigrants recruit better. Back-calculated size at age 1 was unimodal and large (78.2 ± 3.3 mm) in Little Colorado caught fish but was bimodally distributed in Colorado mainstem caught fish (49.9 ± 3.6 and 79 ± 4.9 mm) suggesting that humpback chub can also rear in the mainstem. The study demonstrates the coupled usage of the two rivers by this fish and highlights the need to manage both as a combined system for humpback chub recovery.
Introduction

It is a lost stream, so filthy and muddy that it fairly stinks. It is only 30 to 50 [yards] wide now and in many places a man can cross it on the rocks without going on to his knees ... [The Little Colorado was] as disgusting a stream as there is on the continent ... half of its volume and 2/3 of its weight is mud and silt. [It was little but] slime and salt ... a miserably lonely place indeed, with no signs of life but lizards, bats and scorpions. It seemed like the first gates of hell. One almost expected to see Cerberus poke his ugly head out of some dismal hole and growl his disapproval of all who had not Charon’s pass.

—George Bradley and Jack Sumner, August 1869 [1, p. 234]

The Colorado River of the southwestern US and northwestern Mexico supports critical ecosystem and human services to seven western US states, numerous federally recognized tribes, and several Mexican states. The Colorado River provides water to more than 40 million people, substantial hydroelectric production, and billions of dollars in agricultural products [2]. One of the most striking features of this river basin is Grand Canyon, a UNESCO World Heritage site of cultural, geological, and biological significance protected by the Grand Canyon Protection Act of 1992. President Theodore Roosevelt declared that this is “…the one great site every American should see” and more than 4 million people follow this advice annually to visit Grand Canyon National Park.

The Colorado River in Grand Canyon is an approximately 400-km canyon bound river reach between Lakes Powell and Mead, the two largest reservoirs in the US. Lake Powell, created following the completion of Glen Canyon Dam in 1963, is effectively the primary source of the Colorado River in Grand Canyon regulating the timing, duration, and magnitude of river flows to meet hydropower demands and downstream water release obligations. Lake Powell also retains most of the sediment from the upper Colorado River basin, and both reservoir depth and volume as well as river discharge control water temperatures throughout most of Grand Canyon [3,4]. In the post-Glen Canyon Dam environment, the Colorado River currently transports about <10% of the
historic sediment load (current inputs are from tributaries [5]) and water temperatures are cool (8°C to 10°C from 1994-2002 [6]) with minimal seasonal variation. This is in stark contrast with its undammed state, a strongly seasonally fluctuating environment.

The Colorado River basin historically supported more than 40 endemic fish species and 8 of these species were historically found in Grand Canyon. Of these species, four are thought extinct in Grand Canyon (roundtail chub *Gila robusta*, bonytail chub *G. elegans*, Colorado pikeminnow *Ptychocheilus lucius*, and razorback sucker *Xyrauchen texanus*). Of the remaining fish species, the largest known population of humpback chub *Gila cypha*, a large-bodied, US federally listed (Endangered) cyprinid, is found in nine “aggregations” throughout Grand Canyon, including the largest aggregation near the confluence of the Colorado and Little Colorado rivers. This “Little Colorado River aggregation” likely accounts for more than 90% of the humpback chub in Grand Canyon [7] and the Little Colorado River provides the largest known spawning and rearing habitat for humpback chub in Grand Canyon. Current life history models for humpback chub suggest that adults undertake a potamodromous spawning migration to the unregulated Little Colorado River in the spring, after which they return to the mainstem Colorado River [7-9]. Later in the season, juvenile humpback chub may either (1) emigrate to the mainstem Colorado River as larvae and small juveniles where their survival was thought to be low because of low post-dam water temperatures [10] and predation from non-native species [11] or (2) rear in the Little Colorado River for 1-3 years where growth rates were higher due to warm water and predation risk lower before joining the adult spawning migration [8,9,12]. Based on long-term mark-recapture sampling, adult humpback chub populations in Grand Canyon declined through the 1990’s and early 2000’s [7,9]. The reasons for this decline are unknown [13] but the leading theories include (1) predation by or competition with non-native fish [11,14], (2) loss of required habitats following completion of Glen Canyon Dam related to changes in river flow and sediment supply [15,16], (3) cold, hypolimnetic water releases from Glen Canyon Dam [17,10]. These population declines motivated experimental management actions in Grand Canyon including non-native fish removal [14], translocations to tributary systems to minimize extinction risk [18], and experimental floods to re-build sandbar habitats to stop population declines and aid in population recovery [19].
The Little Colorado River is the largest tributary of the Colorado River in Grand Canyon; is spring fed in the segment of interest (lower 21 km); and receives spring runoff from snowmelt as well as precipitation, typically in summer monsoons. Base flows in the perennially flowing lower 21 km of the Little Colorado River are maintained by springs including Blue Spring, a major upwelling from deep groundwater that brings up carbonated water from a karst aquifer [20]. Upon contact with air, the groundwater degasses, causing supersaturated calcium and magnesium to precipitate as carbonate in travertine (Figure S1) [20-22] among other reactions. As a result, carbon isotopes fractionate as the dissolved inorganic carbon (DIC) in the flowing water equilibrates with the atmosphere and mineralization occurs. This travertine accumulates naturally to create dams in the Little Colorado River that act as barriers to fish migration, restricting humpback chub primarily to the lower 14 km of the Little Colorado River below the large travertine dam that creates Chute Falls [8,16]. Little Colorado River flows vary seasonally, with larger flows occurring during periods of spring runoff and summer monsoon rains.

Previously we presented preliminary data [23] showing that carbon isotopic ratios (expressed as $\delta^{13}C$) in otoliths (earstones) of humpback chub matched $\delta^{13}C$ of end-member waters (the Little Colorado River and the mainstem Colorado River). Briefly, otoliths are calcified structures that form part of the hearing and balance system in teleost (modern) fishes, residing in the inner ear canals. These accrete daily by precipitating aragonite ($\text{CaCO}_3$) on a protein matrix, laying down growth bands similar to tree rings (Figure 1).

Increasingly, the chemical constituents of otoliths are studied to infer environmental histories of fishes [e.g., 24], and when combined with age and growth information, provide powerful means of interpreting fish life histories in spatially explicit contexts [e.g., 23,25]. Essentially, each individual fish carries its own “black box recorder” encoding age, growth, and environmental conditions experienced by the fish.

Here, we develop a more in-depth study of the relationship of otolith chemistry to water chemistry in this system. We then combine otolith chemistry with age and growth information to elucidate humpback chub spatial demography in this region and attempt to address questions related to the provenance and residence of humpback chub in this reach.
of the Colorado River. This is important because controversial, and expensive, management actions are directed at improving habitat conditions for humpback chub in the mainstem Colorado River, but not in the Little Colorado. Specifically, we hypothesize that humpback chub that immigrate as small young-of-the-year (YOY) into the Colorado River are less likely to survive and recruit to the subadult/adult population. Conversely, chub that remain longer in the natal tributary habitat (Little Colorado River), which is warmer and more productive, should grow better and therefore recruit more successfully. Such findings might then lend weight to policy reform to expand the “recognized ecosystem boundaries” for humpback chub to include the Little Colorado River.

**Materials and Methods**

**Ethics Statement** - All water and fish samples were approved by the two entities responsible for managing this land. One is the National Park Service, part of the US Federal Government. These are public lands that are managed by the Park Service. Some of the water samples came from land that is part of the Navajo Nation and is managed by Navajo Nation Fish and Game Department, who also permitted our work.

Otolith extraction can only be humanely done by sacrificing fish, as the structures are located in the skull beneath the braincase. Because of their Endangered status under the US Endangered Species Act [26], directed sampling of large numbers of humpback chub for otolith analyses was not possible. Our samples instead came primarily from incidental mortalities that occurred as part of standard fish monitoring activities by cooperating fisheries research agencies or (for adult humpback chub) fish found dead from unknown causes. A few fish (n < 15 juveniles less than 4 months old) were sacrificed by lethal overdose of anesthetic. Collecting permits for fish and water were obtained from Arizona Game & Fish Department (Scientific Collecting Permit SP790940); U.S. Fish and Wildlife Department Federal Fish and Wildlife Permit (TE212896-0); Navajo Nation Department of Fish and Wildlife (Scientific Collecting Permit 586); and the U.S. National Park Service (Scientific Research and Collecting Permit GRCA-2011-SC1-0041). Animals were handled in accordance with animal
welfare protocols at the University of Florida (IFAS ARC Permit 001-09FAS). All fish samples are in possession of the U.S. Geological Survey’s Grand Canyon Monitoring and Research Center (GCMRC), and were made available to us as a loan for extracting the otolths, after which the bodies were returned to the official repository at the GCMRC.

**Water collection** – Monthly field campaigns were conducted from May 2009 – September 2012. Regular sampling was conducted monthly from July – October, 2009 – 2011, with special collections in May 2009, June 2010, and September 2012. Water samples for trace elemental analyses were allowed to settle so that sediments were removed; then samples were decanted into clean bottles and acidified. Samples for stable isotope analysis were filtered (45 m GF/F) into clean 125-mL bottles, care being taken to leave no head space. All samples were stored in the dark until returned from the field.

**Fish collection** – Fish samples came from the Little Colorado and mainstem Colorado Rivers downstream of the Little Colorado River between river kilometers (rkm) 102-106 (as measured from Lee’s Ferry, AZ) between 1995-2012 with 4 adults available from 1995-2000 and the majority of fish (n = 119) based on sampling since 2000 (Figure 2). The majority of samples were collected during May-October 2009-2011 in both the Little Colorado and mainstem Colorado rivers by sampling for 10-12 days each month for 4 or 5 months each year. In the Little Colorado River, fish collections were made using un-baited hoop nets (50-cm diameter x 100-cm length, 10-cm throat, 6-mm nylon mesh; as in [16] where they were generally fished for 24 hours before being checked for fish captures. In the mainstem Colorado River, similar hoop nets were used and in addition slow-speed boat electrofishing (pulsed DC current, 15-20 amps, 200-300 volts) boat speed 7-10 seconds per meter of shoreline, repeated 24 to 72 hours apart for 3 to 5 total passes per trip) was also conducted. Electrofishing is not possible in the Little Colorado River due to naturally high conductivity.

Lapillar otoliths were dissected from the fish, cleaned, dried, and embedded in epoxy (EpoFix or Epoxicure). Once embedded, frontal plane sections were cut with an Isomet diamond saw (Buehler), polished down to 3 m with successively finer lapping paper (3M). Otoliths that were prepared for secondary ion mass spectrometry (SIMS)
were embedded separately in circular (2.5 cm i.d.) molds, each with 2 pieces of calcite standard (UWC-3 [27]), and polished down to < 1 μm using a powered polishing wheel fitted with diamond-infused polishing cloths. Samples prepared for scanning X-ray fluorescence microscopy (SXFM) were glued to fused quartz glass with cyanoacrylate glue (Loctite). All samples were cleaned by ultrasonication prior to analysis.

**Water chemistry analyses** - Bulk elemental analyses were conducted at the SUNY College of Environmental Science and Forestry Analytical and Technical Services laboratory. Selenium, lead, and zinc were analyzed with inductively coupled plasma mass spectrometry (ICPMS, PerkinElmer Elan DRC-e) in aqueous mode. The remaining major, minor, and trace elements (Ca, Ba, Cu, Fe, K, Mg, Mn, Rb, and Sr) were analyzed via inductively coupled plasma optical emission spectrometry (PerkinElmer Optima 3300DV). Standards were analyzed every 10th analysis; samples that failed QA/QC (> 10% RSD) were re-run until they passed. Only Ca, Ba, Se, and Sr are reported on here.

Water samples were also analyzed for carbon stable isotopic ratios. These were sent to the University of California Davis Stable Isotope Facility where δ^{13}C_{DIC} was analyzed with a Surveyor HPLC coupled to a ThermoFinnigan Delta Plus Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) through a liquid chromatography Isolink interface [28]. Isotopes in dissolved organic carbon (δ^{13}C_{DOC}) were analyzed with an O.I. Analytical Model 1030 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) utilizing a GD-100 Gas Trap Interface (Graden Instruments). To obtain a weighted average of the δ^{13}C_{DIC+DOC}, δ^{13}C values were multiplied by the respective concentrations of DIC and DOC and summed (Table S1).

**Otolith age and growth analysis** – Otoliths were photographed with transmitted light microscopy (40-630X) for measurement and age determination. ImageJ [29] was used to enhance growth bands and make measurements. Annual growth bands (annuli) were counted on optical images or sometimes directly at the microscope; elemental maps were used as supplementary information to help locate annuli. Similarly, daily increments were counted from digital images and were often double-checked at the microscope. Each otolith was read at least twice. In fish that were inferred to have
migrated (as evidenced by a change in otolith chemistry and growth increment width, see below), daily increments were enumerated from the core region (laid down around time of hatch) to the point where the growth bands were too narrow to distinguish, indicating very slow growth. Often such slow growth coincided with a change in otolith chemistry [23].

An otolith length (OL) – fish total length (TL) relationship was developed by measuring the anterior axis of growth, which is the longest one (Figure 1D). The axis becomes curved in age, so a relationship was fit by nonlinear estimation. The best fit was obtained with the following equation:

\[
TL = e^{\left(\frac{OL}{2.7079}\right)^{0.267088}},
\]

\(R^2 = 0.95\). This equation was able to fit the expected values through the smallest individuals (Figure 3).

This equation was used in tandem with otolith chemistry to back calculate fish TL at egress from the Little Colorado River to the Colorado mainstem and corresponding age at egress. A migration event detected in the otolith chemistry (Figure 3) and increment spacing was measured on the otolith and the corresponding size was computed. Total length at formation of the first annulus (corresponding to first “birthday”) was also back-calculated in similar fashion. In this case, a bimodal size distribution was found, and the peaks were tested with the R package “mixdist” (Ichthus Systems [30]).

**Otolith chemistry analyses** – Otoliths were analyzed with any of three methods for this study, and some were analyzed with more than one method for cross-validation (Table 1). Sixty-eight otoliths were analyzed with scanning X-ray fluorescence microscopy (SXFM) at the Cornell University High Energy Synchrotron Source (CHESS) as described in [31,32, 23]. Briefly, trace elements were quantified by stepping a focused beam (10 \(\mu\)m x 20 \(\mu\)m) across the specimen surface with a photon flux of approximately \(10^{11}\) counts per second. Element-specific fluorescences were counted with a Vortex energy-dispersive silicon drift detector fitted with an aluminum foil attenuator to reduce high-intensity calcium fluorescence and increase sensitivity to trace elements.
Calibrations were made using an in-house standard made of finely powdered otoliths pressed into a pellet [32]. Two-dimensional maps of each element of interest were produced by rastering each point for 3 seconds. Data reduction and processing were completed using PyMCA [33] and in-house software developed at CHESS to produce the maps and spatially explicit numerical output (mass fraction). Maps (matrices) of Se, Sr, and occasionally other trace elements were divided by the corresponding Ca map to produce maps of trace element:Ca ratios.

In situ carbon stable isotope analyses were performed using a CAMECA ims-1280 ion microprobe at the WiscSIMS Laboratory (Wisconsin Secondary Ion Mass Spectrometer), UW-Madison. A $^{133}$Cs$^+$ primary ion beam with an intensity of ~600 pA was focused to a diameter of ~8 µm. The typical $^{12}$C$^-$ ion intensity was $7 \times 10^6$ cps, and $^{12}$C and $^{13}$C ions were simultaneously collected by a Faraday Cup detector and an electron multiplier, respectively. Each analysis took ~6 minutes. During each session, 4-6 analyses of the UWC-3 standard ($\delta^{13}$C = -0.91‰ [PDB], [27]) were made before and after each set of 6 – 11 sample measurements. The average spot-to-spot reproducibility or external precision of each set of bracketing standards is ±0.78‰ (2 S.D.). The gain of the electron multiplier was monitored before the third analysis of each group of four standard calcite analyses, and the applied high voltage was adjusted to compensate drift of the gain of the electron multiplier, if necessary. Reproducibility of the $\delta^{13}$C of the bracketing standard analyses includes the drift of the gain of the electron multiplier. A detailed description of the analytical conditions and the instrument setup for carbon isotope measurement has been published previously [34].

A total of 388 $\delta^{13}$C measurements in 16 otoliths were performed, including 182 spots in bracketing standards. Pits placed in domains containing organic matter yield a significantly higher count rate, and data from these pits were excluded from the data set (5 of 206 $\delta^{13}$C measurements in otoliths discarded). A complete table of data is included as online Table S2.

Finally, 37 otoliths were analyzed with laser ablation ICPMS (LA-ICPMS). For this, a New Wave UP193 laser ablation unit was coupled to the ICPMS to ablate solid material from polished otolith sections. The parameters were set to 70% power, 35 µm spot size, 3 µm/s travel time, 10 Hz. The in-house otolith standard as well as a standard
developed by the U.S. Geological Survey, MACS-3 [35], were used both for calibration and to correct for instrument drift. For most runs, transects were set to traverse a pre-defined growth axis (usually ventral or anterior) from the core to the outer edge. In some cases, two transects on different axes were made. Precision on standards was 8-10% for Ca and usually higher for other elements; hence, the data were smoothed with a 5-point interval. Seven minor or trace elements (Ba, Cu, Mg, Mn, Na, Sr, and Zn) as well as Ca could be quantified.

Migratory status was determined by whether a marked change was observed in otolith chemistry (Figure 3, see above). The putative first emigration from the Little Colorado River to the mainstem was identified both by the chemical signature on the otolith as well as a marked narrowing of daily growth increments [23]. This latter was due to movement into colder water, which slows both somatic and otolith growth. Temperature, rather than food availability, most strongly affects the circadian pattern of daily increment deposition [36], so much so that hatcheries routinely batch mark otoliths by manipulating temperatures slightly upward to induce a broad growth band or downward to induce a narrow band [37]. Thus, the combination of shifts in otolith chemistry and narrowing of daily growth increments was interpreted as emigration from the Little Colorado River to the mainstem.

Results

Water chemistry – Water sampling over the three years of study revealed differences between the geochemistry of the Little Colorado River vs. the mainstem (Table 2, Figure 4). Barium, selenium, and strontium in ratio to calcium were lower (but not statistically different ($p > 0.05$)), and $\delta^{13}C$ was higher ($p < 10^{-6}$), in the Little Colorado River. Importantly, the variability of the Little Colorado River chemical parameters was far greater than was the case for the mainstem Colorado. Examination of the data (Figure 4) shows that Little Colorado River Ba:Ca, Se:Ca, and Sr:Ca were lower on 8, 11, and 9 of 13 sampling events, respectively, and $\delta^{13}C$ was higher on all but one of 11 sampling events ($\delta^{13}C$ analysis was not done in July – September 2009). Most of the carbon was in the form of DIC, but this fraction was larger ($0.97 \pm 0.01$ S.E., $N = 10$) in the Little Colorado River than in the mainstem ($0.89 \pm 0.01$ S.E., $N = 9$). The difference
between $\delta^{13}C$ at the two sites averaged $9.3 \pm 1.2 \%$ (S.E.) (Table S1), with Little Colorado being higher.

Because the monsoonal season for the region occurs in summer to early fall and generates flashy precipitation events in the Little Colorado River well above its base flow (Figure S2), high flow events can rapidly and radically alter chemical conditions there. The discharge of the mainstem, on the other hand, is far more constant as it is controlled release water from Lake Powell and is 2-4 orders of magnitude greater than that of the Little Colorado River. The trace Sr:Ca and Ba:Ca ratios in the Little Colorado River were inversely proportional to the fraction of a given month with flows less than threshold values of 6.5, 8, and 10 $m^3/s$ (used as proxies for base flow and near base flow), and $\delta^{13}C$ was positively, though less strongly, correlated with these thresholds (Figure S3). Little relationship was shown between Se:Ca ratios and thresholded flow frequencies (Figure S3). These results suggest that high flow events tend to release more strontium and barium from the watershed, and that the same events tend to dilute the travertine $\delta^{13}C$ with meteoric water. The high variability in the relationships also suggests that the concentrations may depend on which part of the watershed becomes wetted in specific flow events. However, we note that Ba:Ca and Sr:Ca were lowest, and $\delta^{13}C$ highest, when flows were $< 10 m^3/s$. Given that over the study period, the Little Colorado River daily discharge was less than $10 m^3/s$ 84% of the time, we conclude that differences are large enough between the two rivers to discern distinct differences that would appear in otolith chemistry. We also note that water sampling was conducted during monsoonal months and thus disproportionately sampled during or shortly after high flow events; on the other hand, the humpback chub live continuously in the environment, incorporating ambient chemistry of whichever river they are in at the time.

Otolith chemistry – Carbon stable isotopic ratios in the otoliths showed patterns consistent with residency in both the Little Colorado River and the mainstem Colorado River. Small (< 30 mm) fish collected at Boulders Camp in the Little Colorado River, 3 km upstream of its confluence with the mainstem, had mean $\delta^{13}C$ values of $-2.5\%\pm 0.6$ (95% confidence interval) and did not differ significantly from values in migrant fish that were classified as residency in the Little Colorado River ($-3.2\%\pm 0.6$). The values for
portions of migrant fish otoliths identified as residency in the mainstem were considerably depleted in $^{13}$C ($\delta^{13}$C = -11.3‰ ± 0.6). The difference between the otolith mainstem signature and the mean of the two Little Colorado River signatures (-2.9‰) is 8.3‰, remarkably close to the difference observed in water from the two sources.

The change in $\delta^{13}$C(otolith) from Little Colorado River to mainstem signature in otoliths tended to be abrupt, and could occur on the order of a day or two (Figure S4). In contrast, trace elemental shifts tended to be more gradual (Figure 5). A linear regression of Sr:Ca on $\delta^{13}$C was significant ($R^2 = 0.53$, $p < 10^{-4}$). Ba:Ca (outside of the core, see below) was also inversely related to $\delta^{13}$C but the relationship was more scattered ($R^2 = 0.15$, $p < 0.1$).

Despite the weak relationship of trace elements to $\delta^{13}$C, in some individual fish there were strong concordances among trace elements. For example, specimen HBC-3D9 had strong concordance in Sr, Se, and Ba (Figure 6). We interpret the peaks in these elements as residency in the mainstem, and lower values as residency in the Little Colorado River. In contrast, sodium, which is high in the Little Colorado River [38], is elevated during this fish’s first growing season (summer), drops in the first winter, rises in the second summer, drops in the second winter, and then stabilizes (Figure 6B, right panel). We interpret this as further evidence of the fish spending the first two summers in the Little Colorado River and the first two winters in the mainstem. We hypothesize that the lack of dynamic pattern in the remainder of the Na:Ca transect may be due to physiological stabilization of ionic regulation, despite further movements between the two rivers.

A number of otoliths had elevated concentrations of several trace elements in their core regions (Figure 7). These appear to be located in primordia or nucleation points within the core; a number of otoliths had visible multiple primordia. Most were detected with LA-ICPMS as SXFM is not sensitive to some of the elements due to interferences or low mass number. Up to five different trace elements (Ba, Cu, Mg, Mn, and Zn) were simultaneously detected as elevated in cores, often with a concomitant drop in Sr concentration. Of 32 otoliths for which core analysis was of sufficient quality (i.e., the core was intact and exposed at the specimen surface), 7 contained all five elements.
17 showed peaks in Ba, Mg, and Mn (“Group 2”), 2 had peaks in Ba, Mg, Mn, and either Cu or Zn (“Group 3”), and 6 had other combinations including only Mg, Mg and Mn, Ba and Cu, and a single individual had a sodium spike and no other elevated metals (“Group 4”). Analysis of variance revealed no effect on groups of capture location or year of birth; however, very few were captured away from the vicinity of the Little Colorado River/mainstem region.

Otolith-derived estimates of demographic events – 77 otoliths were assessed for size at egress into the mainstem Colorado River. Back-calculated size at emigration ranged from 9.5 to 107.4 mm TL with a mean of 46.1 mm ± 2.1 S.E. The smallest-sized migrant had 19 broad daily increments, followed by a notable check and smaller daily increments, but eventually broadening out again even while the δ¹³C values remained depleted (Colorado River signature; Figure S4). Size at egress differed significantly (p < 0.001) depending on whether the fish was a young-of-the-year or older. Mean size at egress of fish < 1 year at capture was 35.5 mm ± 3.6 s.e. (N = 25) whereas fish ≥ 1 year averaged 51.0 mm ± 2.2 s.e. (N = 52; Figure 8A). Generally, most individuals’ growth rates decelerated markedly upon entry into the mainstem, reflected in very tight otolith growth bands (see [23]).

Age at egress determinations were made on 65 fish. Age at first egress averaged 79.7 ± 6.1 (S.E.) days, though one was estimated to have been approximately 250 days old and emigrated at the end of its first winter. As with length at egress, age at egress differed significantly between fish less than or ≥ 1 year at capture (p < 0.001; Figure 8B). Young-of-the-year that emigrated were on average 51.2 ± 4.4 days at egress (N = 37); fish 1 year and older were 100 ± 7.8 days old at egress (N = 28). Age and length at first egress were strongly correlated and were fit with a linear regression (Age at egress = -7.95 + 1.96 (TL at egress), R² = 0.61, p < 10⁻⁴ (Figure S5).

Total length at first annulus formation (Age 1) varied between the two rivers (Figure 9). Specifically, length at Age 1 was unimodal in the Little Colorado River-caught fish, bimodal in the mainstem-caught fish, and bimodal overall. Mean length at Age 1 for fish captured in the Little Colorado River was 78.2 ± 3.3 mm. Modes for the CO-caught vs. overall bimodal distributions were close (Table 3).
We were able to assess provenance and recent residency of 110 humpback chub from 15-408 mm TL (Figure 10). We found that 95% of the fish we assessed were born in the Little Colorado River and the remainder from an unidentified location. Of these 110 fish, 25% (N = 28) were born in the Little Colorado River, then used the mainstem Colorado River for some portion of their life, and subsequently returned to the Little Colorado River where they were captured. An additional 48% (N = 53) fish were born in the Little Colorado River and remained in the mainstem after egress while 21% (N = 23) were born in the Little Colorado River and remained there. The remaining 5% (N = 6) were not clearly identifiable with respect to provenance. Based on examination of growth patterns it appears that these fish were most likely born in the mainstem in a location that is atypical in terms of chemistry and temperature to the surrounding water such as a warm spring location [23].

Discussion

Water chemistry in the mainstem Colorado River was characterized by constancy, whereas our sampling of water chemistry in the Little Colorado River showed considerable variability as it transitioned between its “blue water” baseflow to its turbid, high flow states. This variability was driven by high flow events that were disproportionately sampled relative to their actual frequency of occurrence in the Little Colorado River. When accounting for flow frequency, water chemistry differed sufficiently between the Little Colorado and mainstem Colorado rivers to provide reliable end member markers that could be taken up and quantified in humpback chub otoliths.

Of the markers investigated, δ^{13}C discriminated most strongly between rivers and was faithfully reflected in otoliths. Carbon stable isotope ratios become elevated in the Little Colorado River due to degassing of deep groundwater as it bubbles out of springs in the system, precipitating travertine at the same time. As is typically the case in lotic travertine systems [22], δ^{13}C increased downstream from Blue Spring (Limburg et al., in preparation). Carbon isotopic uptake in humpback chub otoliths was rapid; we detected changes of up to ~ 9‰ on the order of 1-2 days in closely-spaced spot measurements which suggests that carbon signatures should exist in otoliths to delineate spatial location of a fish within a few days of the fish moving from the Little Colorado River to mainstem.
or vice-versa. In an experimental study, $^{14}$C uptake from labeled ambient DIC was measurable in goldfish *Carassius auratus* otoliths as soon as one hour following incubation [39]. These investigations also found that 75% of the carbon incorporated in otoliths came from DIC with the remainder being metabolic; this was similar to the findings of 80% DIC derived otolith carbon in rainbow trout *Oncorhynchus mykiss* [40]. Metabolic C contributed 35 – 45% of otolith C in bluegill sunfish *Lepomis macrochirus* that were exposed to a whole-lake addition of $^{13}$C and the change in lake chemistry was transmitted to the otoliths in a few days [41]. Similarly, we assume that most of the C taken up by humpback chub otoliths derives from the DIC rather than from their food; however the food sources should also be isotopically labeled by the DIC [42].

Although $\delta^{13}$C is a superior tracer of fish provenance and movement in this system, in practice it is a difficult and costly analysis, both in water and otoliths. We therefore sought [23; this study] complementary tracers that are easier and more cost-effective to use. In this regard, strontium:calcium (Sr:Ca) ratios appear to be the most reliable choice. This as well as other trace elemental markers (Ba:Ca, Se:Ca) in chub otoliths appear to lag by perhaps 1-2 weeks relative to carbon stable isotopes, and the transition is more gradual as well. Thus, we over-estimate slightly our age and size at emigration when using trace elemental markers alone; however, this overestimation should be evenly distributed amongst emigrants. Others have also noted the gradual incorporation of Sr:Ca into otoliths [e.g., 43].

Sodium is another potential tracer of juvenile humpback chub residency in the Little Colorado River. This is referred to as a “salt river,” and Hopi Indian traditions include a pilgrimage along the Salt Trail [44]. Although present in moderately high concentrations in otoliths, sodium has generally been assumed to be under such great physiological control that it would serve little value as a provenance tracer [45]. However, the very high concentrations of sodium in the Little Colorado River may cause an increase of otolith Na:Ca during the juvenile period, when growth is rapid. Hoff and Fuiman [46] found a significant decline in Na:Ca ratios in juvenile red drum *Scianops ocellatus* exposed to increasing salinities. The relationship of high dissolved sodium to otolith Na:Ca doubtless is constrained by physiology and phylogeny (drum are scianids and chubs are minnows), but bears further investigation in humpback chub.
In a similar vein, we found that a number of different trace elements were highly concentrated in the primordia of chub otoliths. Manganese has often been cited as under maternal control as its presence in otolith cores has been found across different taxa [e.g., 47,48] but not in all species [e.g., 32]. In the present study, we found that 7 different elements could “spike” in the otolith core, and up to 5 of them more or less simultaneously. However, careful examination of lasered specimens showed that spikes of different elements could occur in adjacent primordia (Figure 7). It is likely that primordia chemistry is determined by maternal deposition of trace elements in eggs. Future work should further explore the causes and whether there are patterns of geographic variation within the Colorado River system; for example, eggs could be collected from females in different parts of the Grand Canyon and analyzed for trace elements.

How does an understanding of these chemical tracers and otolith growth of humpback chub help in our understanding of the population ecology of this species? We suggest two key findings: (1) age and size at egress of juvenile humpback chub from the Little Colorado River into the mainstem differed between young-of-the-year and older individuals, and (2) size at age-1 of humpback chub differed between fish captured in the Little Colorado River vs. the mainstem. Little Colorado River humpback chub size at age-1 was distributed about a unimodal peak, whereas mainstem fish had a bimodal peak (Figure 8). The larger of the bimodal peaks from the Colorado River matched closely the mode of the distribution of fish from the Little Colorado River. This suggests that this larger of the two modes observed in the mainstem may represent fish that remained in the Little Colorado River for a longer period of time before emigrating to the Colorado River.

We found that most of the young-of-the-year humpback chub (27 of 37) we assessed were estimated to emigrate from the Little Colorado River during June-August\(^1\), when Little Colorado River flows can be at some of their lowest levels of the year depending on when monsoon rains begin. As an example, of these 37 fish that were

\(^1\) Most of the August departures were in 2009, when flows were close to that of June and July. August discharges can be considerably higher due to monsoons.
captured in the mainstem that we were able to estimate month of egress, 20 of these came from 2009. When daily flows in the Little Colorado River are examined for 2009 (USGS gauge 09402300), values from May-December are generally similar between 6-8 m$^3$/s, with three small monsoonal storm peaks (May 24 at 23.9, July 21 at 11.2, and September 14 at 15 m$^3$/s). Of the 20 fish in our sample that emigrated from the Little Colorado River to the mainstem, 8 emigrated in August, 4 each in June and July, 3 in May, and 1 in April – all under low flow conditions. Granted, our sampling was uneven and constrained by permit requirements, so we cannot state that this is a dominant behavior. Nevertheless, it demonstrates that emigration is not necessarily dependent on flood pulses, and that juvenile humpback chub may emigrate based on some other cue, potentially humpback chub density in the Little Colorado River or some other ecological or environmental phenomenon.

Our findings suggest a size advantage at their first birthday for fish that remain in the Little Colorado River for longer time periods, and that this size advantage may persist when these fish egress into the mainstem Colorado River. For humpback chub, survival is thought to be strongly influenced by the fish age and size and this relationship is assumed in the key stock assessment framework developed for this species [9]. Larger body size is known to offer many survival advantages including improved winter survival related to higher lipid accumulation [49], greater range of prey resources available [50], and improved swimming ability and predator avoidance [51] ultimately potentially leading to improved fitness for these surviving individuals [52–54]. All of these advantages may be important for juvenile humpback chub to survive in the mainstem Colorado River under current conditions. In the post-Glen Canyon Dam environment, invertebrate fish prey resources may be altered such that fish now must broaden their diets [55] even while prey availability declined. Additionally, cold water temperatures may impair swimming ability for smaller fish [10, 17], leading to increased predation risk from non-native predators [11]. These survival constraints could be mitigated by larger size at egress from the Little Colorado to Colorado rivers.

Growth patterns of juvenile humpback chub (these same fish) were documented from tag recaptures in the Little Colorado and mainstem Colorado River in response to experimental flow retreatments in the mainstem [56 and unpublished data]. Based on
growth rates estimated from tag-recaptured fish, in order to maximize growth, juvenile humpback chub should spend spring and summer in the Little Colorado River and emigrate to the mainstem Colorado River during winter when growth declined. Evidence was found of juvenile humpback chub moving from the Little Colorado River, to the mainstem, and then returning to the Little Colorado River from otolith microchemistry [23] and this has also been observed from tag recaptures (W. Pine, personal observation). This type of movement pattern would potentially maximize growth from a temperature perspective but it is unknown if prey availability follows the same spatial and temporal pattern between the Little Colorado and mainstem Colorado Rivers. In our current study, this behavior was rare amongst juveniles (< 3%); it may be necessary to increase the sample size, including the spatial and temporal distribution of samples, to obtain a more reliable perspective on these types of fine-scale movement patterns between these systems. This study is based primarily on incidental mortalities which may introduce unknown bias into our interpretation. However, given the Endangered status of humpback chub, the risk to the population from directed sampling of larger numbers of humpback chub was unknown. This risk is now better quantified and can be considered for future directed sampling for humpback chub otoliths to address additional research and management questions [18].

While it is well documented from tagging studies that adult humpback chub migrate between the mainstem Colorado River and Little Colorado River for spawning [57], movement patterns of juveniles between the mainstem and Little Colorado rivers prior to spawning is not as well studied and has been difficult due to low capture probabilities of tagged fish and limited sampling in the mainstem Colorado River for juvenile fish. Collectively our results, including Hayden et al. (2012), suggest that juvenile humpback chub can and do utilize both the Little Colorado and mainstem Colorado rivers. Additionally, ongoing work suggests a mechanistic framework for why it may be thermally advantageous for juvenile fish, in terms of improved growth and survival [56], to use both rivers. This is important because management efforts have been directed at improving conditions in the mainstem Colorado River to promote survival of juvenile humpback chub. These efforts include experimental flows to improve survival [56], modify habitats [58,59], and removals of non-native species...
In contrast, limited conservation actions have been made in the Little Colorado River since critical habitat designation [60]. In view of the fact that special areas such as Blue Springs and other groundwater sources still warrant designation 20 years afterwards [61,62], additional conservation and protection of the Little Colorado River appears justified.

We cannot definitively address the robustness of our geochemical atlas across seasons, but rather must make inferences about the applicability of our collected data to other times of the year. That said, the weight of the evidence within humpback chub otoliths, in terms of combined chemistry and growth patterns [36], supports our contention that the markers are valid for assigning fish emigrant status. Together with retrospective estimates of size and age at key events (first egress and first birthday), our results support the hypothesis that chub remaining in the Little Colorado longer than ca. 2 months have improved chances of recruitment, and that those remaining even longer in the Little Colorado River grow larger by Age 1 and are thus better conditioned for survival in the mainstem Colorado. Depending on whether additional, systematic sampling of humpback chub occurs, other questions could be investigated, such as whether winter residency in one or the other river enhances or retards growth, how many winters are spent in one vs. the other river, what proportion of chub located in the Little Colorado River are true residents of that area, etc.

The largest question – how important is the Little Colorado River to humpback chub? – is answered easily as “very important.” There is limited evidence for successful spawning in other locations in this river reach other than the Little Colorado River [23,63] and the risk to this population from some sort of catastrophic event in the Little Colorado River has motivated management agencies to develop a series of actions to conserve this species including translocation efforts to other tributaries [64]. Additionally the key management guidelines for this species [65] specify coordinated efforts by conservation interests to expand research assessing the role of tributaries in contributing to the resilience of humpback chub populations. Our results help to provide a tool to meet this goal by creating a framework for assessing the contribution of recruits from the mainstem Colorado River and from tributary systems with different water chemistry signals. This information could be extremely useful in prioritizing
conservation efforts for developing secondary spawning populations of humpback chub
as specified in recovery documents [65].

Conclusions

Our results confirming the use of the Colorado River as juvenile rearing habitat,
coupled with the persistence of these juvenile cohorts for multiple years [56] suggests
that, at least in recent years, the mainstem Colorado River has been suitable for juvenile
humpback chub. This is a significant finding. It is likely that this rearing capacity, in
addition that of the Little Colorado River [61] is a potential reason for the observed
increases in juvenile recruitment and ultimately adult humpback chub documented in the
Little Colorado River aggregation in recent years [66,61]. Overall this work clarifies the
important role the mainstem Colorado River in Grand Canyon likely plays in the
recovery of humpback chub populations.

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preparation, D. Dale for assistance with SXFM analyses, and T. Gushue for preparation
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Summit Logistics for technical support, and C. Fritzinger for logistical support. Any use
of trade names is for descriptive purposes only and does not imply endorsement by the
U.S. Government.

Author Contributions

Conceived and designed the study: KL, TH, WP. Performed the study: KL, TH, RK, JV.
Analyzed the data: KL, WP, TH, MY. Contributed reagents/materials/analysis tools: JV,
RK. Wrote the paper: KL, WP, TH, MY, RK, JV.
References


List of Figures

Figure 1. Collage of humpback chub and their lapillar otoliths. Older, larger fish have larger, more curved otoliths. Clockwise from left center: A – otolith of a 20 mm post-larva with 32 daily growth increments; B – otolith from a 2 year old, 114-mm subadult; C – subadult humpback chub (Photo: C. Finch); D – otolith from a 7 year old, 230-mm adult, with line drawn along the axis of measurement; E – old humpback chub (Photo: C. Finch); F – otolith with laser ablation track from a 28 year old, 383-mm chub. Bar on otolith images = 100 microns.

Figure 2. Map of study area, showing Little Colorado River from its confluence with the mainstem of the Colorado River up to Blue Spring, the source of elevated δ¹³C.

Figure 3. Illustration of back-calculation of size at first egress. Top figure is a strontium map of a humpback chub otolith; below it is the total length-otolith length nonlinear fit. The distance on the longest growth axis (anterior) to a migration event (elevated Sr) is measured; the corresponding total length is computed using Equation 1.

Figure 4. Water chemistry and discharge data. A. Comparing water chemistry in the mainstem just above Little Colorado River, and Little Colorado River at Boulders, 2009 – 2011. Each year has a single value for July – October.

Figure 5. A. Concordance of trace elements Sr:Ca and Ba:Ca to δ¹³C in an otolith. Carbon isotopic ratio shifts abruptly with habitat shift, whereas trace elements shift more gradually. B. Regression of Sr:Ca on δ¹³C.

Figure 6. A. Trace elemental maps generated by SXFM. Note the concordance of Sr and Se and the elevated Cu in the core region. B. Trace elemental transects of Sr, Ba, and Na in ratio to Ca measured with LA-ICPMS. Note qualitative similarities of Sr:Ca and Ba:Ca to the patterns in the trace elemental maps, and the inverse relationship of Na:Ca to Sr:Ca up to approximately 550 microns.

Figure 7. Otolith from a 25-mm, 30-day-old humpback chub captured in the Little Colorado River, June 2010. Note the presence of three primordia (arrows) that appear to be elevated in Ba, Mn, and Mg respectively. Dotted line shows direction of laser track but its width is not to scale.
Figure 8. Frequency histograms of back-calculated humpback chub size (A) and age (B) at egress from the Little Colorado River, separated by age at time of capture.

Figure 9. Back calculated lengths at first annulus formation for humpback chub captured in the Little Colorado (Little Colorado River) vs. Colorado (CO) rivers.

Figure 10. Number of humpback chub (y-axis) by size (x-axis) demonstrating specific residency patterns as determined from otolith microchemistry or growth analyses. “Other/MS” blue bars represent fish that were born in an unknown location, but then determined to be living in the mainstem Colorado River (MS). “LCR/MS” labeled orange bars are fish that were born in the Little Colorado River and then emigrated to the mainstem Colorado River at some point in their life. The “LCR/LCR” green bars are fish that were born in the Little Colorado River and remained in the Little Colorado River.

Supplemental Figures.

Figure S1. Photo of travertine formations in Little Colorado River. Credit: C. Finch.

Figure S2. Discharge characteristics of the Little Colorado River near its confluence with the mainstem Colorado River, May 2009 – December 2012. A. Hydrograph. Lines represent the 6.5, 8, and 10 m$^3$/s levels of discharge. Note the flashy storm hydrographs interspersed among very low base flow conditions. Data from U.S. Geological Survey National Water Information System (http://waterdata.usgs.gov/nwis). B. Flow frequency curve for Little Colorado River. C. Temporal patterns of flow frequencies less than 6.5 (blue), 8 (red), and 10 (green) m$^3$/s in Little Colorado River.

Figure S3. Linear regressions of (A) barium:calcium ratios, (B) strontium:calcium ratios, (C) selenium:calcium ratios, and (D) $\delta^{13}$C, all vs. the percent of discharges within a month that was less than specified threshold values of 6.5, 8, and 10 m$^3$/s.

Figure S4. Example of an otolith showing positions of ion microprobe ablations and corresponding $\delta^{13}$C data. The fish was collected on 23 July, 2010 in the mainstem; it
was 24 mm TL and 63 days old. Note the rapid shift from elevated (Little Colorado River) to $^{13}$C-depleted values.

Figure S5. Humpback chub total length (mm) vs. age (days) at egress from the Little Colorado River to the mainstem Colorado River.
**Table 1.** Numbers of individual fish used for different otolith chemistry analyses.

<table>
<thead>
<tr>
<th>SXFM</th>
<th>SIMS</th>
<th>LA-ICPMS</th>
<th>N</th>
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<tbody>
<tr>
<td>X</td>
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<td>51</td>
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<td></td>
<td>X</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total 97</td>
</tr>
</tbody>
</table>

**Table 2.** Means (± s.e.) of barium, selenium, and strontium relative to Ca, and δ\(^{13}\)C collected over 3 years (May 2009 – October 2011).

<table>
<thead>
<tr>
<th>River</th>
<th>Ba:Ca x 10^{-3}</th>
<th>Se:Ca x 10^{-3}</th>
<th>Sr:Ca x 10^{-3}</th>
<th>δ(^{13})C, ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Little Colorado River</td>
<td>1.38 (0.36)</td>
<td>0.019 (0.002)</td>
<td>9.34 (0.92)</td>
<td>0.59 (1.24)</td>
</tr>
<tr>
<td>Colorado mainstem</td>
<td>1.43 (0.03)</td>
<td>0.024 (0.002)</td>
<td>10.80 (0.08)</td>
<td>-8.87 (0.49)</td>
</tr>
</tbody>
</table>

Notes: Little Colorado River includes a special collection made in June 2010, which investigated longitudinal variation in water chemistry of that river. δ\(^{13}\)C is a weighted average of DIC and DOC. Little Colorado River trace elements N = 14 events; Colorado mainstem elements N = 13 events; Little Colorado River δ\(^{13}\)C N = 10; Colorado mainstem δ\(^{13}\)C N = 9.
Table 3. Results of bimodal analysis of back-calculated total lengths at Age 1.

A. All fish:

<table>
<thead>
<tr>
<th>Mode</th>
<th>p_i</th>
<th>Mu</th>
<th>sigma</th>
<th>Chi^2</th>
<th>Pr(&gt;Chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.324 (0.11)</td>
<td>50.4 (3.51)</td>
<td>6.71 (2.68)</td>
<td>0.215</td>
<td>0.99</td>
</tr>
<tr>
<td>2</td>
<td>0.676 (0.11)</td>
<td>80.4 (2.97)</td>
<td>10.24 (2.21)</td>
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<td></td>
</tr>
</tbody>
</table>

B. Colorado mainstem-caught fish:

<table>
<thead>
<tr>
<th>Mode</th>
<th>p_i</th>
<th>Mu</th>
<th>sigma</th>
<th>Chi^2</th>
<th>Pr(&gt;Chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.507 (0.15)</td>
<td>49.9 (3.59)</td>
<td>6.874</td>
<td>0.59</td>
<td>0.90</td>
</tr>
<tr>
<td>2</td>
<td>0.492 (0.15)</td>
<td>79.04 (4.94)</td>
<td>9.496</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p_i = proportion of data falling under the mode; Mu = modal mean; sigma = spread.

Standard errors in parentheses.
Figure 2
The graph shows a scatter plot with a trend line given by the equation:

\[ TL = \exp((OL/a)^b) \]

The coefficient of determination, \( R^2 = 0.95 \), indicates a strong correlation between the variables. The x-axis represents the otolith core-to-tip length (microns), while the y-axis shows the total length (mm).
Figure 4
A

B

Mean individual Sr:Ca x 1000

Mean individual $\delta^{13}C$, ‰

$Sr:Ca = 2.9361 - 0.2082 \times \delta^{13}C$
Figure 6.
Figure 7
Figure 8
Figure 9.
Figure 10.

Figure 10.
Supplemental Information – Limburg et al.

**Supplemental Figures.**

Figure S1. Photo of travertine formations in Little Colorado River. Credit: C. Finch.

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Figure S5. Humpback chub total length (mm) vs. age (days) at egress from the Little Colorado River to the mainstem Colorado River.

**Supplemental Tables.**

**Table S1.** Carbon isotopic ratios ($\delta^{13}$C, ‰) and C concentrations (ppm) in dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and concentration-weighted average $\delta^{13}$C at Boulder Camp, most downstream sampling site in the Little Colorado River, and the mainstem Colorado River upstream of the Little Colorado. Absolute difference in $\delta^{13}$C between the two sites is computed for co-occurring dates.

**Table S2.** *In situ* carbon isotope analysis by SIMS (Secondary Ion Mass Spectrometry).
Figure S2
Figure S-2, continued
Figure S3

$y = -2.629x + 3.3002$

$R^2 = 0.2511$

$y = -4.4805x + 5.1726$

$R^2 = 0.3729$

$y = -5.9677x + 6.7481$

$R^2 = 0.344$

Percent of Monthly $Q < $ Threshold Value

$< 6.5 \text{ m}^3/\text{s}$

$< 8 \text{ m}^3/\text{s}$

$< 10 \text{ m}^3/\text{s}$
Figure S3, continued
Figure S3, continued
Figure S3, continued

Percent of Monthly Q < Threshold Value

\[ y = 3.8786x - 0.6709 \quad R^2 = 0.195 \]

\[ y = 4.7754x - 2.0241 \quad R^2 = 0.1458 \]

\[ y = 8.2912x - 5.2148 \quad R^2 = 0.219 \]

< 6.5 m³/s

< 8 m³/s

< 10 m³/s
Figure S4.
Age at egress = -7.9535+1.9607*x

Figure S5.
Supplemental tables.

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<table>
<thead>
<tr>
<th>Date</th>
<th>Site name</th>
<th>ppm DIC δ13C (PDB, ‰)</th>
<th>ppm DOC δ13C (PDB, ‰)</th>
<th>Fraction as DIC</th>
<th>Weighted Mean δ13C, ‰</th>
<th>Difference between Little Colorado River and MS, ‰</th>
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<tr>
<td>May-09</td>
<td>Boulder Camp</td>
<td>132.82 1.35 2.87 -2.37</td>
<td>0.979 1.28</td>
<td>9.47</td>
<td>Oct-09 Boulder Camp</td>
<td>78.58 3.69 3.38 -7.40</td>
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<tr>
<td>Jun-10</td>
<td>Boulder Camp</td>
<td>84.18 2.11 1.88 -7.42</td>
<td>0.978 1.90</td>
<td>10.47</td>
<td>Jul-10 Boulder Camp</td>
<td>61.99 5.35 0.66 -11.33</td>
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<td>Aug-10</td>
<td>Boulder Camp</td>
<td>77.38 0.42 3.08 -20.45</td>
<td>0.962 -0.38</td>
<td>8.78</td>
<td>Sep-10 Boulder Camp</td>
<td>70.94 1.15 3.22 -22.51</td>
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<td>Boulder Camp</td>
<td>76.48 2.12 0.07 -23.54</td>
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<td>10.53</td>
<td>Jul-11 Boulder Camp</td>
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<td>Aug-11</td>
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<td>Date</td>
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<td>Depth</td>
<td>Temperature</td>
<td>Salinity</td>
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<td>Departure</td>
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<td>Sep-11 Boulder Camp</td>
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Mean 0.97 0.59 9.31
S.E. 0.01 1.24 1.15

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Depth</th>
<th>Temperature</th>
<th>Salinity</th>
<th>Arrival</th>
<th>Departure</th>
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<tr>
<td>May-09 Mainstem</td>
<td>63.18</td>
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Bracket (standard analyses 248-251, 260-263) \(-43.5\)

±2 SD of bracketing standard analyses \(0.7\)

(10) Sample change: Otolith # 20101026 EF04

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Bracket (standard analyses 248-251, 260-263) \(-43.5\)

±2 SD of bracketing standard analyses \(0.7\)
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Bracket (standard analyses 269-273, 272-275)

± 2 SD of bracketing standard analyses

0.7

(11) Sample change: Otolith # 201024 HP05

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Bracket (standard analyses 278-281, 290-293)

± 2 SD of bracketing standard analyses

0.6

(12) Sample change: Otolith # 201022 HP05

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Bracket (standard analyses 306-310, 303-305)

± 2 SD of bracketing standard analyses

0.7

Bracket (standard analyses 319-322, 318-322)

± 2 SD of bracketing standard analyses

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<td>$\delta^{13}C$ [% PDB]</td>
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<td>$\delta^{13}C$ [measured]</td>
<td>2 SE</td>
<td>$^{12}C$ [10^4 cps]</td>
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<td>-2.8</td>
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<td>±2 SD of bracketing standard analyses</td>
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<tr>
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<td>0.56</td>
<td>7.3</td>
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<td>Bracket (standard analyses 402-405, 414-417)</td>
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<td>±2 SD of bracketing standard analyses</td>
<td>0.6</td>
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Column [A]: Carbon isotope ratios in PDB scale, corrected for instrumental bias. The UWC-3 calcite standard has a calibrated $\delta^{13}C$ of -0.91‰ PDB [Kozdon et al., 2009]
Column [B]: ±2 SD of the bracketing standard analyses
Column [C]: Raw measured $^{13}C/^{12}C$ ratios
Column [D]: Internal error of a single analysis calculated as the twice of the standard error of the mean of 20 cycles
Column [E]: Secondary intensity of $^{12}C$ ions in counts per second (cps)

Reference:
USING OTOLITH CHEMISTRY TAGS AND GROWTH PATTERNS TO DISTINGUISH MOVEMENTS AND PROVENANCE OF NATIVE FISH IN THE GRAND CANYON

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b Hammond Bay Biological Station, Great Lakes Science Center, Millersburg, Michigan, USA
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ABSTRACT

Fish otolith and water chemistry were assessed in the Grand Canyon reach of the Colorado River and its tributaries. Aqueous strontium and selenium (in ratio to calcium) and carbon stable isotopic ratios were identified as markers with excellent potential to track the provenance and movements of the endangered humpback chub Gila cypha. Although otolith δ13C and Sr/Ca varied proportionately to water chemistry and provided a framework for detailed study of humpback chub movements, otolith Se/Ca showed ambiguous tracking of known water chemistries. As an application, we document the natal source and movement dynamics of n = 10 humpback chub and compare these findings from otolith microchemistry with the current paradigm of humpback chub spawning ecology. We found that seven of ten fish follow the current early life history paradigm and were spawned in the Little Colorado River and subsequently emigrated to the main stem Colorado River as juveniles. However, the otolith markers of three fish suggest an alternative early life trajectory with unknown provenance. Age and growth analyses demonstrate seasonally higher growth rates in the warmer Little Colorado River compared with the Colorado River. Combining natural markers with age and growth reconstructions provides a powerful tool for assessing the habitat use and success of humpback chub in the Grand Canyon. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS: otolith chemistry; humpback chub; Grand Canyon; fish growth/age; Colorado River

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INTRODUCTION

The Grand Canyon reach of the Colorado River is an internationally recognized cultural and ecological treasure (UNESCO status 1979). Since 1963, this canyon-bound river reach has been highly influenced by operation of the Glen Canyon Dam. Completion of the dam created physical ecosystem changes, including attenuation of floods, reductions in sediment inputs, daily water level fluctuations due to hydropower production and relatively constant, cool water temperatures (Howard and Dolan, 1981; Stanford and Ward, 1991; Stevens et al., 1997; Schmidt et al., 1998; Topping et al., 2008). Concurrent biological effects include changes in food web dynamics across all trophic levels (Stevens et al., 1997; Shannon et al., 2001; Cross et al., 2011), including the introduction of several non-native fish species (Rinne and Janisch, 1995; Nico and Fuller, 1999; Olden and Poff, 2005), as well as a reduction or total loss of successful spawning and rearing conditions for native fishes in the Colorado River main stem (Robinson et al., 1998; Gorman and Stone, 1999).

Currently, only four of the endemic species historically found in Grand Canyon still exist there today. One of these, humpback chub Gila cypha, has received protection as an endangered species under the US Endangered Species Act (ESA) since the Act was enacted in 1973. The largest humpback chub population is located in Grand Canyon near the confluence of the Colorado and Little Colorado rivers (Valdez and Ryel, 1995; Coggins et al., 2006). In addition to the humpback chub population in Grand Canyon, five other humpback chub populations are known and all are found in the Colorado River basin. Management actions intended to enhance humpback chub populations include experimental flow regimes (Cross et al., 2011) and removal of non-native predators (Coggins et al., 2011). Although the numbers of adult humpback chub in the Grand Canyon population have increased in recent years, species persistence is thought to be dependent on a single spawning tributary (Little Colorado River). Research efforts continue to assess the mechanisms limiting humpback chub recovery (Coggins et al., 2006, 2011; Yard et al., 2011) and are used for assessment and development of management actions undertaken for species remediation.

Within Grand Canyon, the humpback chub population is structured as a series of nine discrete aggregations. The largest aggregation is located in the Colorado River near
the confluence of the Little Colorado River (rkm ~100, as measured downstream of Lee’s Ferry, Arizona; Coggins et al., 2006). The Little Colorado River is a spring-fed tributary that is characterized by extensive travertine (i.e. calcium carbonate) riverbed deposition. Although perennial water flows in the Little Colorado River are supported by a large spring complex located approximately 21 km from the confluence with the Colorado River, only the lower ~14 km of the river are colonized by humpback chub owing to a natural barrier waterfall and naturally occurring high dissolved carbon dioxide levels (Robinson et al., 1998).

Tagging studies on humpback chub have been ongoing since the mid-1980s and have provided a general framework for tracking adult fish movements between the Little Colorado River and the Colorado River. The resulting data suggest that the Little Colorado River and Colorado River habitats are linked by seasonal potamodromous spawning migrations of adult humpback chub. Adult Individuals move from the Colorado River to the Little Colorado River in March to June for spawning, returning to the main stem after spawning (Gorman and Stone, 1999). As part of this tagging program, the minimum size for tagging humpback chub has been 150 mm total length (TL) or approximately age 4 years (Coggins et al., 2006), and in recent years, the minimum size at tagging has shifted to 100 mm TL (ages 2 or 4 years). However, very little information is available on the movement dynamics of smaller, younger humpback chub because they are below the minimum size for Passive Integrated Transponder (PIT) tagging. The current paradigm of early life humpback chub ecology suggests that after hatching in the Little Colorado River, larval and juvenile humpback chub emigrate to the Colorado River at different life stages and times (Gorman and Stone, 1999). Hypotheses for the decline of humpback chub populations in Grand Canyon include (1) changes in water temperature and sediment (Converse et al., 1998; Clarkson and Childs, 2000); (2) non-native fish interactions (Yard et al., 2011); and (3) non-native parasites (Hoffnagle et al., 2006). Given the importance of early life ecology on population persistence and that the survival of emigrating humpback chub is thought to differ with fish size and age, juvenile humpback chub are increasingly the focus of research efforts and management actions, including the removal of rainbow trout (Coggins et al., 2011; Yard et al., 2011), the experimental flow releases from Glen Canyon Dam and the translocations of juvenile humpback chub from the Little Colorado River to other tributaries within Grand Canyon (B. Healey, Grand Canyon National Park, Flagstaff, Arizona, personal communication). The goals of these management actions include establishing humpback chub populations within the Colorado River and establishing refuge populations in other tributaries. As it contains approximately 95% of the Grand Canyon population of humpback chub (Kaeding and Zimmerman, 1983; Coggins et al., 2006), the Little Colorado–Colorado River confluence region is of critical conservation importance for the persistence of humpback chub and the recovery of this unique species.

The other Grand Canyon humpback chub aggregations consist of small pockets of fish that are not known to spawn successfully (but see Andersen et al., 2010 and references therein), although the aggregations persist and are consistent in their presence and relative abundance across years and sampling events (Paukert et al., 2006). Of these aggregations, only one is found upstream of the Little Colorado River near a spring complex that flows directly into the Colorado River (rkm 48), whereas the remaining aggregations are located downstream of the Little Colorado confluence.

A key uncertainty in our knowledge of humpback chub ecology is related to the source and fates of juvenile humpback chub. Given the cold water and non-native predator densities in the main stem, the survival of larval and small juvenile humpback chub emigrating from the warmer, unregulated Little Colorado River is thought to be lower than later emigrants due to reduced growth and increased predation risk (Valdez and Ryel, 1997; Robinson et al., 1998; Robinson and Childs, 2001). However, recent assessments have shown an increasing trend in the Grand Canyon humpback chub population (Coggins et al., 2006; Coggins and Walters, 2009) and these increases could be from the increased production of humpback chub and or the increased survival of juvenile humpback chub. If so, are these increases solely coming from Little Colorado River–spawned fish or do other unknown spawning areas exist? Is the Grand Canyon population of humpback chub supported only by fish whose provenance is the Little Colorado River? If so, this would further emphasize the need for strong conservation of the Little Colorado River to reduce the risk of extinction due to catastrophic events within the Little Colorado River watershed.

In this study, we begin to address this uncertainty by characterizing and identifying site-specific water and humpback chub otolith chemistry markers in the Grand Canyon. We then use these otolith chemistry markers to identify humpback chub fish provenance and migration trajectories to inform our knowledge of the spatial ecology of juvenile and adult humpback chub. Multiple trace elements and carbon stable isotopes were evaluated as potential natural markers. These naturally occurring otolith “tags” (suites of incorporated trace elements and isotopes) are increasingly used to study fish migrations and provenance (Elsdon et al., 2008). Given that some trace metals and isotopes become incorporated in the calcium carbonate structure of the otolith in relation to their presence in ambient water, otoliths eliminate the need to artificially mark fish for future recaptures. Otolith tags also permit the analysis of larval and juvenile fish that are too small to bear artificial tags. Furthermore, fish otoliths are chronometric structures that are present before egg hatching and grow throughout the life of the fish.
such that analyses of otolith chemistry can provide lifetime trajectories of an individual fish’s movements, provided that these migrations include waters with different chemistries (Campana and Thorrold, 2001; Weidel et al., 2007). When chemistries are combined with traditional age and growth analysis, the two approaches may yield great insights into how specific habitats affect fish growth and ultimately survival.

METHODS

Humpback chub \((n = 10)\) were obtained from collections maintained by the US Geological Survey (USGS) Grand Canyon Monitoring and Research Center (GCMRC) in Flagstaff, Arizona for otolith chemistry investigations. Given the endangered species status of humpback chub, specimen collection is highly regulated and all fish used in this study were incidental mortalities during research and monitoring activities of different cooperating agencies in Grand Canyon. Fish used in this study represent individuals collected from three of the known humpback chub aggregations in Grand Canyon. We chose these aggregations to represent a range of possible provenance and life history trajectories. In the Colorado River, fish were available from the 30-Mile Spring aggregation \((\sim 48\text{ rkm})\), the Colorado River–Little Colorado River confluence aggregation \((\sim 100\text{ rkm})\) and the final 12 km of the Little Colorado River aggregation (Table I; Figure 1). Of the ten fish included in this study, eight individuals in the collection were small and were presumed to be juveniles, whereas two individuals were larger adults or subadults (Kaeding and Zimmerman, 1983; Table I).

Lapillar otoliths were selected for otolith chemistry analyses and were removed via dissection and cleaned of any adhering organic matter by immersion in a dilute \((10\% \text{ v/v})\) bleach–water solution. Preparation of otoliths for microchemical analyses was adapted from Secor et al. (1991). A single otolith was randomly chosen for elemental analyses and was cast into rectangular molds using EpoFix (Struers) cold-set epoxy. Epoxy blocks were sectioned in the frontal plane through the core with a low-speed diamond saw (Buehler, IsoMet) and then polished using progressively finer grades of aluminium oxide lapping film until the otolith core was exposed, as determined by bright-field light microscopy. Polished otoliths were subsequently mounted on fused-quartz glass slides using cyanoacrylate adhesive (Loctite). Immediately before elemental analyses, all samples were ultrasonically cleaned in deionized water. Otoliths were photographed at magnifications of \(200 \times \) to \(630 \times\) and the daily rings were counted without prior knowledge of the identity (i.e. the ID code or collection location) of the fish. For the two larger fish, ages in years were determined, and daily rings deposited from hatching during the juvenile phase were enumerated until growth slowed so much that daily rings could no longer be discerned.

Table I. Collection dates, locations and lengths and ages at capture for humpback chub used in this study

<table>
<thead>
<tr>
<th>Fish no.</th>
<th>Collection date</th>
<th>Collection location</th>
<th>rkm</th>
<th>TL (mm)</th>
<th>Age</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>20 August 2009</td>
<td>COR</td>
<td>101</td>
<td>33</td>
<td>140 days</td>
</tr>
<tr>
<td>2</td>
<td>May 2003</td>
<td>LCR</td>
<td>1.1</td>
<td>37</td>
<td>1+ year</td>
</tr>
<tr>
<td>3</td>
<td>24 September 2006</td>
<td>COR</td>
<td>64.6</td>
<td>19</td>
<td>83 days</td>
</tr>
<tr>
<td>4</td>
<td>24 September 2006</td>
<td>COR</td>
<td>64.6</td>
<td>18</td>
<td>na</td>
</tr>
<tr>
<td>5</td>
<td>25 September 2006</td>
<td>COR</td>
<td>78.1</td>
<td>21</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>June 2010</td>
<td>LCR</td>
<td>1.6</td>
<td>27</td>
<td>33 days</td>
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<tr>
<td>7</td>
<td>June 2010</td>
<td>LCR</td>
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<td>28</td>
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<td>8</td>
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<td>LCR</td>
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<tr>
<td>10</td>
<td>7 October 2009</td>
<td>LCR</td>
<td>9</td>
<td>255</td>
<td>5+ years</td>
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COR, Colorado River; LCR, Little Colorado River; rkm, river kilometre (for COR, downstream of Lee’s Ferry, Arizona; for LCR, upstream of LCR-COR confluence); TL, total length.

Figure 1. Map of the Colorado River and its tributaries in the Grand Canyon. The largest extant humpback chub population is located near the Colorado River and Little Colorado River confluence (circled). Numbers denote GCMRC river kilometres from Lee’s Ferry, Arizona.
Multiple otolith trace elemental concentrations were quantified using scanning X-ray fluorescence microscopy at the F3 Beamline Station at the Cornell High Energy Synchrotron Source. Scanning X-ray fluorescence is a spectral technique that uses high-energy X-rays to produce an elemental fluorescence spectrum. A double-bounce multilayer monochromator provides a 16.1-keV incident beam with 0.6% bandpass. A single-bounce glass capillary was used to focus the incident beam to a 20-μm (horizontal) by 10-μm (vertical) spot at the sample with a photon flux of approximately $10^{11}$ counts per second (Bilderback et al., 2003; Cornaby, 2008). Two-dimensional surface maps of elemental concentrations were created by stepping the beam across the entire surface of the sample in a sequential, non-overlapping grid pattern. At each step, the fluorescence spectrum was integrated for 3 s before moving to the adjacent sample location. Fluorescence X-rays were detected with a Vortex energy-dispersive silicon drift detector fitted with an aluminium foil attenuator to reduce high-intensity calcium fluorescence and increase sensitivity to trace elements. Initial spectral processing consisted of screening for a suite of 25 trace elements. However, only Se, Sr and Ca concentrations exhibited consistent variation between and within fish. Samples are reported as molar ratios to Ca (millimole element per mole Ca; Campana, 1999). Instrumental calibration was achieved using an in-house standard reference material consisting of ground otolith material of known trace elemental concentrations (Limburg et al., 2011). Data reduction and processing were completed using PyMCA (Solé et al., 2007) and in-house software developed at Cornell High Energy Synchrotron Source to produce two-dimensional elemental maps and spatially explicit numerical output. Numerical data were imported to a geographic information system to extract sequences (i.e. transects) of elemental concentrations from the two-dimensional maps (Quantum GIS Development Team, 2011). All elemental sequences extended from the otolith core to the otolith edge, parallel to the longest growth axis of the otolith.

Given that otolith and water chemistry are correlated for many trace elements and isotopic ratios, Sr/Ca, Se/Ca and carbon stable isotopic ratios ($\delta^{13}\text{C}$) in both dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) were quantified at multiple locations within the Grand Canyon during a 21-day research expedition in October 2009. In addition to water samples collected in the Colorado River, major tributaries were sampled to quantify the influence of tributary chemistry on the Colorado River chemistry and identify tributaries with unique water chemistries for the assessment of potential humpback chub spawning and rearing sites.

Fifteen locations were sampled within the Colorado River at sites located immediately upstream and downstream of major tributary confluences. Additionally, samples were collected from eight major tributaries (Paria River, Little Colorado River, Nankoweap, Bright Angel, Shinumo, Tapeats, Havasu and Diamond creeks) near their confluence with the Colorado River and one spring that flows directly into the Colorado River was sampled (30-Mile Spring). At all sample locations, two separate samples were collected for quantifying trace elemental concentrations and $\delta^{13}\text{C}$ stable isotope ratios. For trace elemental analyses, 500 mL polyethylene bottles containing 3 mL of high-purity nitric acid (OPTIMA) were filled by submerging the bottle in the water (Eaton and Franson, 2005). Samples for dissolved inorganic and organic $\delta^{13}\text{C}$ isotopic ratios were filtered in the field using a syringe fitted with an in-line filter enclosure containing a glass microfilter filter (filter enclosure, Pall; filter, Whatman GF/F, 25 mm diameter). Filtered water was collected in 50 mL screw top centrifuge vials such that no bubbles or headspace were present or introduced when collecting sample. All samples were stored in darkness and on ice until completion of the sampling trip. Immediately following the conclusion of the research expedition, samples for DIC and DOC $\delta^{13}\text{C}$ isotopic ratio analyses were shipped to the University of California-Davis Stable Isotope facility for analysis using isotope ratio mass spectrometry. Concentrations of trace elements were determined at State University of New York College of Environmental Science and Forestry using inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin-Elmer Optima 3300DV) and inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Elan DRC-e 6100). Strontium and calcium were quantified using ICP-OES and Se was quantified using the ICP-MS outfitted with a dynamic reaction cell set to monitor $^{80}\text{Se}$. Instruments were calibrated using external standards and monitored for drift by analyzing quality control samples after every 10 unknown samples. For a run to pass quality controls, all quality control samples had to be within ±10% of known values. All water samples were above detection limits for all reported analytes. Multivariate trends in water chemistry were visualized using a principal component analysis of the correlation matrix.

In addition to analyses of trace elements in water and otoliths, in situ carbon stable isotope measurements of one juvenile humpback chub otolith were conducted using the CAMECA IMS 1280 secondary ion mass spectrometer instrument at the Northeast National Ion Microprobe Facility (Woods Hole, Massachusetts). This instrument is a large radius, double-focussing mass spectrometer fitted with an ion detection system consisting of two Faraday cups and a single electron multiplier. The secondary ion extraction system consisted of a Cs+ ion beam combined with a high-energy normal-incidence electron gun for charge compensation. Ions were extracted by rastering the beam over the

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1The $\delta^{13}\text{C}$ denotes the ratio of rare and heavy ($^{13}\text{C}$) to abundant, light ($^{12}\text{C}$) isotopes, relative to the same ratio in a standard; in this case Pee Dee Belemnite (PDB). By convention the PDB $\delta^{13}\text{C}=0$.  

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surface of the sample in a 30-μm × 30-μm pattern. Estimates of δ¹³C and the precision of the estimates were calculated by bracketing unknown otolith analyses with analysis of an in-house standard reference material (Carrara marble). Results are reported as per mille against the PDB standard. Values of δ¹³C may take on negative or positive values.

The humpback chub otolith was analyzed at one location near the otolith core and again near the otolith’s edge. As with scanning X-ray fluorescence microscopy analyses, the humpback chub otolith was embedded in epoxy, cut in a frontal plane and polished with progressively smaller diamond grits until the otolith core was exposed at the surface (final polish = 0.05 μm diamond suspension). The secondary ion mass spectrometer instrument sample holder accepts 25.4 mm round samples and, as such, the embedded otolith with standard reference grains were pressed into a round (25.4 mm diameter) indium metal plug. In addition to providing a secure method for mounting the sample in the instrument, the indium disk also minimizes background interferences. Immediately before analysis, samples were ultrasonically cleaned in deionized water and sputter-coated with gold.

RESULTS AND DISCUSSION

Spatial trends in water chemistries

Water chemistries of the Colorado River and tributaries in Grand Canyon were spatially and temporally heterogeneous for Sr/Ca, Se/Ca, DOC δ¹³C and DIC δ¹³C across sites and sample dates (Table II). Compared with the Colorado River, tributary water chemistry exhibited substantially more variability (Table II). Similarly, for all elemental and isotope ratios quantified, the range of values measured was substantially greater in the tributaries than those measured in the Colorado River (Table II). Inspection of vector loading plots from principal component analysis revealed that dimension 1 is positively correlated with DIC and DOC δ¹³C and dimension 2 is correlated with Sr/Ca and Se/Ca (Figure 2a). Overall, the first two principal components accounted for 84% of the variance in the data set (dimension 1 = 59%, dimension 2 = 25%; Figure 2a). The water chemistry of the Colorado River was similar throughout the entire Grand Canyon and tightly grouped in principal component analysis (PCA) biplots (Figures 1 and 2b). Lake Powell is a large reservoir and serves to homogenize the water chemistry at the beginning of the Grand Canyon reach of the Colorado River. The consistency of water chemistry throughout the Grand Canyon suggests that tributary inputs to the Colorado River are small and quickly diluted. With the possible exception of Paria River and Nankoweap Creek, all tributary multivariate water chemistry signatures were readily distinguished from the main stem Colorado River (Figures 1 and 2b). Comparing tributary water chemistry, Bright Angel, Tapeats and Shinumo creeks were similar but had lower trace element and isotopic elemental ratios compared with the Colorado River (Figures 1 and 2b). The Little Colorado River, Havasu Creek and 30-Mile Spring had water chemistries that were characterized by high δ¹³C values and low element to calcium ratios compared with the Colorado River chemistry (Figures 1 and 2b).

Juvenile (and putatively juvenile) humpback chub otolith chemistry and growth

Tracking fish migrations with otolith chemistry requires validated site-specific otolith chemistry signatures (Elsdon et al., 2008). Site-specific natural tags have been assessed by quantifying the otolith chemistry of juvenile humpback chubs, after testing on a proxy species (flannelmouth sucker Catostomus latipinnis; Hayden et al., in prep.). Although recent emigration to the collection location presents the possibility that otolith chemistry does not match that of the capture point, it is less likely that small juvenile fish (e.g. <35 mm) have emigrated long distances and thus represent signatures from a different site. Given that the largest humpback chub population in the Grand Canyon is located within the Little Colorado River and nearby in the Colorado River (Coggins et al., 2006), we have focussed on identifying otolith markers that distinguish fish

Table II. Water elemental and isotopic ratios for the Colorado River and tributary streams, October 2009

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<tr>
<th></th>
<th>Sr/Ca (mmol-mol⁻¹)</th>
<th>Se/Ca (mmol-mol⁻¹)</th>
<th>δ¹³DOC, ‰ (PDB)</th>
<th>δ¹³DIC, ‰ (PDB)</th>
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</tbody>
</table>

Tributaries include Paria and Little Colorado rivers and Nankoweap, Bright Angel, Shinumo, Tapeats, Havasu and Diamond creeks. COR, Colorado River; Trib, tributaries; Mean, mean water chemistry; SD, standard deviation of water chemistry; N, number of samples; Min, minimum value; Max, maximum value.
residency in these habitats. In this way, fish with markers that do not match the Little Colorado River can be identified as fish that were spawned or reared in a previously unknown location. This is of significant conservation interest because, if observed, it would suggest that spawning takes place outside of the Little Colorado River.

To validate our assumptions related to the incorporation of identifying water characteristics into the fish otoliths, the otolith trace elemental chemistry of three resident Little Colorado fish was quantified. Fishes 6 to 8 (Table I) were collected in the Little Colorado River as juveniles (~25 mm TL) upstream from the Little Colorado–Colorado River confluence (Figure 1). Given their small size and capture locations, it is likely that these fish originated in the Little Colorado River and are not recent immigrants. Trace element otolith chemistry transects between the otolith core and edge were characterized by constant Sr/Ca ratios, suggesting that water chemistry was temporally stable during the time period in which these fish inhabited the system (Figure 3, fishes 6–8). These otoliths provide a ‘known’ Sr/Ca signature for the Little Colorado River. Daily growth rings in these fish were very clear and large (Figure 4, top panels).

In addition to analysis of resident Little Colorado River humpback chub otoliths, we quantified Sr/Ca ratios in a 33-mm humpback chub collected in the Colorado River near the Little Colorado River confluence (Table I, fish 1). The otolith core to edge transects revealed that Sr/Ca ratios doubled between the otolith core (Sr/Ca = 0.5 mmol/mol) and edge (Sr/Ca = 1 mmol/mol; Figure 3, fish 1). Core Sr/Ca ratios in fish 1 were similar to the Sr/Ca ratios measured in the resident Little Colorado River fish (fishes 6–8), suggesting that this fish originated in the Little Colorado River. Given that water Se/Ca and Sr/Ca ratios are lower in the Little Colorado River than that in the Colorado River and that this fish was collected in the Colorado River, the higher Sr/Ca ratios at the otolith edge (i.e. portion of otolith formed immediately before capture) is consistent with a migration to the Colorado River (Figure 3, fish 1).

In addition to quantifying otolith trace element chemistries, fish 1 was also analyzed for carbon stable isotope ratios at its core and outer edge (Table I; Figure 3). In this

Figure 2. Multivariate principal component analysis of Colorado River and tributary water chemistry in the Grand Canyon. Water Sr/Ca, Se/Ca, DOC δ13C and DIC δ13C were quantified. Samples were collected in October 2009. Variable loadings (a) and biplot (b) of the first two principal components. Solid squares represent samples collected in the Colorado River, open circles are tributary samples and the open square is the spring sample. Circles depict sample sites with similar chemistry (solid, Bright Angel Creek, Tapeats Creek and Shinumo Creek; dashed, Little Colorado River, Havasu Creek and 30-Mile Spring; dot-dashed, Colorado River, Paria River, Diamond and Nankoweap creeks).

Figure 3. Otolith Se/Ca and Sr/Ca transects measured in juvenile humpback chub otoliths using scanning X-ray fluorescence. Transects started at the otolith core and extended to otolith edge, parallel to the growth axes of otoliths. Arrows in pane 1 denote the locations of secondary ion mass spectrometer analyses depicted in Figure 5. See Table I for biological characteristics of fish.
fish, the $\delta^{13}C$ value measured at the otolith core and edge was approximately $-1\%$ (PDB) and $-13\%$ (PDB), respectively (Figure 5). The otolith core $\delta^{13}C$ is a close match for values measured in the Little Colorado River water, whereas the $\delta^{13}C$ values measured at the outer edge of the otolith are a close match for Colorado River water. The difference between otolith core and edge $\delta^{13}C$ in fish 1 is substantial ($-13\%$) and as such, we confirm the results from the trace elemental chemistry that this individual originated in the Little Colorado River and moved to the Colorado River where it was collected at an early age. Furthermore, outgassing of mantle-derived carbon dioxide with a high $\delta^{13}C$ value ($-5\%$) in the Blue Spring (Figure 1, 21 km upstream of the confluence of the Little Colorado River and the main stem) is well documented and supports the observed differences in otolith chemistry between otolith core and edge in fish 1 (Crossley et al., 2006, 2009). As such, this individual provides information concerning the characteristic otolith Sr/Ca signature of the Colorado River.

The age and growth history of fish 1 corresponded well with otolith chemistry. From hatching (estimated to be at the beginning of April), there were 37 large, distinct daily growth increments (through early May), followed by 59 very small increments (i.e. into early July), followed by a period of such slow growth that we could only estimate the number of growth rings as 40 to 45. This last period...
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The otolith chemistry of fish 5, collected in the Colorado River upstream of the Little Colorado River, had a unique Sr/Ca chemistry compared with all other fish in this study. As with fishes 3 and 4, this individual was collected downstream (rkm ~78) of the 30-Mile Spring as a 21-mm TL individual in September 2006 and was aged at 75 days (Table I). The Sr/Ca transect between the otolith core and edge of this fish was characterized by a constant, low Sr/Ca ratio (Figure 3). In fact, the constant chemistry between the otolith core and edge and the fact that its chemistry was lower than any other fish in the study suggest that this fish was not spawned in the Little Colorado River. The low otolith chemistry of this fish does not match the typical main stem Colorado River chemistry signature that all other fish analyzed to date have incorporated into their otoliths. Given our knowledge of water chemistry in the system, this fish likely spawned in a previously unknown site within the Colorado River and exhibited high site fidelity to this location. However, fish 5’s daily growth increments were very narrow, suggesting cooler water conditions in the unknown nursery habitat as compared with the Little Colorado or 30-Mile Spring nurseries.

In contrast to the consistent patterns in Sr/Ca observed in the otolith core–edge transects, Se/Ca transects were

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inconsistent for seven of eight juvenile humpback chub examined (Figure 3). Otolith Se/Ca ratios in fish 1 increased between the otolith core and edge from approximately 0.002 to 0.0035 mmol-mol$^{-1}$. On the basis of water chemistry, Sr/Ca ratios and $\delta^{13}$C, the observed increase of Se/Ca ratios between the otolith core and edge in fish 1 is consistent with a migration between the Little Colorado River and the Colorado River. Within an individual, the Se/Ca ratios of fishes 2 to 8 varied between otolith core and edge but transect trends were not consistent across individuals with similar collection locations and migratory histories as identified using otolith Sr/Ca, otolith $\delta^{13}$C or water chemistry. We have observed more variability in aqueous Se/Ca during flooding events (Hayden et al., in prep.), which may partly explain the variability. Additionally, physiological interactions of Se with metals such as mercury could potentially affect Se uptake (Lochet et al., 2010). Mercury concentrations were not quantified in this study and as such, the role of mercury remains unknown in terms of affecting Se/Ca in these juvenile otoliths.

**Larger humpback chub otolith chemistry**

Fishes 9 and 10 were larger humpback chub and their otolith chemistry transects exhibited multiple Sr/Ca and Se/Ca peaks between the otolith core and edge (Figure 6). In both fish, the maximum Sr/Ca ratio observed in the transect was located approximately 40% to 60% of the distance between the core and edge with subsequent Sr/Ca peaks decreasing toward the otolith edge (Figure 6). Although the maximum Sr/Ca ratios in fishes 9 and 10 are higher than observed in fishes 1 to 8, this may represent interannual variation in site-specific otolith chemistry owing to basin-wide processes such as Glen Canyon Dam releases or discharge within the Little Colorado River. Temporal shifts in otolith chemistry are well documented when comparing site-specific otolith chemistries across multiple years (Gillanders, 2002). Given our observations of high Sr/Ca ratios with Colorado River residency and low Sr/Ca with Little Colorado River residency, the multiple peaks in the adult humpback chub chemistry between otolith core and edge likely represent multiple fish movements between the Little Colorado River and the Colorado River (Figure 6).

Otolith Se/Ca ratios also increased between otolith core and edge in the adult humpback chub. In the largest adult fish included in the study (fish 10, TL = 255 mm), Se/Ca peaks were positively correlated with Sr/Ca, as expected by water chemistry analyses (Figure 6). In fish 9, the correlation between otolith Sr/Ca and Se/Ca was observed but was not as strong.

Fish 9 was more than 1 year old and fish 10 was more than 5 years old (Figure 7). Otolith daily increments deposited

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*Figure 6. Otolith Se/Ca and Sr/Ca transects of larger humpback chub using scanning X-ray fluorescence. Transects started at the otolith core and extended to the otolith edge, parallel to the longest growth axis of the otolith. See Table I for biological characteristics.*
during the first growing season were visible out to 138 and 176 days, respectively. We note the stark contrast between fish 9 (112 mm) and fish 2. The latter fish, originally thought to be a 37-mm juvenile but with more than 140 visible daily increments and a capture date of May, is likely either a more than 1-year-old fish spawned in the spring or a fall-spawned fish. Further analysis will reveal whether or not such small fish actually recruit to the adult population.

CONCLUSIONS AND RECOMMENDATIONS
In conclusion, strong discrimination of the Little Colorado River from the Colorado River by carbon stable isotopes measured in water was observed in otolith chemistry and provides a distinct marker for fish movements between these two systems. Furthermore, results of water sampling efforts throughout the Grand Canyon suggest that the carbon stable isotope ratios in the Little Colorado are unique, although several tributaries (e.g. Havasu Creek) and 30-Mile Spring show some similarity (Limburg et al., in prep.). Although more subtle, differences in otolith trace elements, specifically Sr/Ca ratios, can also resolve fish movements between the Colorado River and the Little Colorado River. Our results suggest that incorporation of Se/Ca in otoliths may be influenced by hydrological events (and perhaps by concentrations of other elements such as mercury) and thus may not be a reliable natural marker, although further study is needed.

In this system, otolith chemistry may be an effective tool for identifying fish migration trajectories, source-sink dynamics, provenance or dispersal. We have shown that linking otolith chemistry and microstructure provides important information concerning the timing of fish movements and resulting growth patterns that may be important for future management of the Grand Canyon humpback chub population.

On the basis of our results, are we able to resolve any of the uncertainties related to movement dynamics of juvenile native fish? The fish used in this study are not random samples from the population but instead, as incidental mortalities from ongoing monitoring, represent available samples of juvenile humpback chub from three of the known
aggregations of humpback chub within the Grand Canyon population. Thus, absolute estimates of spawning contributions from different areas in the Grand Canyon are not possible at this time. However, our results do confirm the current spawning ecology paradigm for this species by highlighting the importance of the Little Colorado River as the primary spawning location. Our results also suggest that other spawning may be occurring in the main stem in the 30-Mile Spring region and that some larvae that spawn in this region may persist in the main stem Colorado River beyond the immediate 30-Mile Spring area. A larger random sample of humpback chub juveniles across multiple size classes and different spatial locations would be required to further assign the probabilities of these different natal origin and rearing life history types and, ultimately, what proportion of the adult recruits use, and especially spawn, in this area outside of the known Little Colorado River spawning aggregation.

Our most significant result is the documentation of otolith microchemistry methods to delineate the natal origins of juvenile humpback chub in Grand Canyon. This is a key result going forward; humpback chub populations increased during the first decade of the 2000s (Coggins et al., 2006; Coggins and Walters, 2009), but the mechanisms for this increase are unknown. Increases in population size can result from either increase in birth rate or decreases in death rate. Understanding which of these is occurring in Grand Canyon is critical for determining what management actions are necessary to aid in the recovery of the species. Recent management actions have focused on the removal of non-native predators (primarily rainbow trout Oncorhynchus mykiss) in an effort to reduce mortality on juvenile humpback chub (Coggins et al., 2011; Yard et al., 2011). Although humpback chub populations have increased during this period of reduced predator abundance, apparently due to increases in recruitment of juveniles (Coggins and Walters, 2009), whether this was related to the removal of non-native fish or other factors influencing recruitment is still uncertain (Coggins et al., 2011; Yard et al., 2011). Because of the recognized need to conserve and enhance native fish populations in the main stem Colorado River, understanding the source or sources of these recruits is important to understanding the overall population dynamics of humpback chub in Grand Canyon. If the Little Colorado River continues to be the only suitable spawning location in terms of recruitment success, then the recovery of humpback chub in Grand Canyon will be limited to the spawning and rearing carrying capacity of the Little Colorado River—further highlighting the critical need for conservation to protect the Little Colorado River as a critical humpback chub habitat to ensure long-term viability of this species in Grand Canyon. With a larger sample of juvenile humpback chub that reflects spatial and temporal considerations, the increased statistical power should make these questions amenable to answers.

ACKNOWLEDGEMENTS

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REFERENCES


Results of Water Chemistry Sampling of the Colorado River and Tributaries in Grand Canyon, 2009-2012

Introduction

In order to support our otolith chemistry investigations, we sampled and analyzed water from several sites along the Colorado River as well as key tributaries. Our initial studies cast a fairly broad net in terms of analytes, but eventually these were narrowed down to some extent, as were the sampling sites themselves.

Methods

Elemental Analysis

Single or duplicate water samples for major, minor, and trace elements were collected by bucket. Silt was allowed to settle out before the water was decanted into flasks containing ultrapure nitric acid (bringing it to a concentration of approx. 1 mL/L HNO₃) to maintain ions in solution. All samples were stored in the dark until returned from the field.

In some cases, particularly during August-October 2011, samples were not allowed sufficient time to settle. This affected the concentrations of certain trace elements (e.g., Mn, Pb). In the case of the most important trace elements for otolith chemistry (Ba, Se, Sr) this did not present a problem because their ratio to calcium was preserved. However, some values are flagged in the Excel file by being shaded in gray.

A final water collection was conducted in September 2012 in which all water was filtered through glass fiber filters (0.7 micron pore size).

Bulk elemental analyses were conducted at the SUNY College of Environmental Science and Forestry Analytical and Technical Services laboratory. Selenium, lead, and zinc were analyzed with inductively coupled plasma mass spectrometry (ICPMS, PerkinElmer Elan DRC-e) in aqueous mode. The remaining major, minor, and trace elements (Ca, Ba, Cu, Fe, K, Mg, Mn, Rb, and Sr) were analyzed via inductively coupled plasma optical emission spectrometry (PerkinElmer Optima 3300DV). Standards were analyzed every 10th analysis; samples that failed QA/QC (＞10% RSD) were re-run until they passed.
Stable Isotope Ratio Analysis

Duplicate samples for stable isotope analysis were filtered (0.7 μm GF/F) into clean 125-mL bottles, care being taken to leave no head space. All samples were stored in the dark until returned from the field. Samples were initially collected for oxygen and hydrogen/deuterium stable isotopic ratios. However, in October 2009, we also assayed for carbon stable isotopic ratios and discovered that $^{13}\text{C}$ proved to have the best discriminatory power between the mainstem and the Little Colorado River (the major spawning tributary of humpback chub). At that point, the May 2009 samples were retroactively analyzed for $^{13}\text{C}$ but the samples for July, August, and September 2009 were not available. Analysis of $^{13}\text{C}$ was conducted thereafter, and oxygen and H/D isotopic ratio analyses were only done occasionally.

Samples for carbon stable isotope analysis were sent to the University of California Davis Stable Isotope Facility where $\delta^{13}\text{C}_{\text{DIC}}$ was analyzed with a Surveyor HPLC coupled to a ThermoFinnigan Delta Plus Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) through a liquid chromatography Isolink interface [28]. Isotopes in dissolved organic carbon ($\delta^{13}\text{C}_{\text{DOC}}$) were analyzed with an O.I. Analytical Model 1030 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) utilizing a GD-100 Gas Trap Interface (Graden Instruments). To obtain a weighted average of the $\delta^{13}\text{C}_{\text{DIC+DOC}}$, $\delta^{13}\text{C}$ values were multiplied by the respective concentrations of DIC and DOC and summed.

Samples analyzed for $^{18}\text{O}$ and D/H were sent to the Center for Stable Isotope Biogeochemistry at the University of California at Berkeley. Both analysis types are made by isotope ratio mass spectrometry. As described by the website (http://nature.berkeley.edu/stableisotopelab/), $^{18}\text{O}$ in water is analyzed by continuous flow (CF) using a Thermo Gas Bench II interfaced to a Thermo Delta Plus XL mass spectrometer. We use the method described in the Thermo GAS Bench II operating manual, ThermoQuest, Oct 1999. In brief, 20-200 μL of water (depends on the sample volume available) for both standards and samples are pipetted into 10 mL glass vials (Exetainer®, Labco Ltd., UK) and quickly sealed. The vials are then purged with 0.2% CO2 in
Helium and allowed to equilibrate at room temperature for at least 48 hours. The 18O in the CO2 is then analyzed. Long-term external precision is ± 0.12‰.

“D/H in water is analyzed in dual inlet (DI) using a hot chromium reactor unit (H/Device™) interfaced with a Thermo Delta Plus XL mass spectrometer. We add multiple standards to every run and we correct for differential drift of standards with different isotope ratios. Long-term external precision ± 0.80‰.”

Results

The data are presented in Excel spreadsheets that are available for download here http://floridarivers.ifas.ufl.edu/NSE.htm

Each spreadsheet contains elemental concentrations as well as elements in ratio to calcium.
Habitat Selection and Movement of Sub Adult Humpback Chub in the Colorado River in Grand Canyon, Arizona during Experimental Steady Flow Releases

Brandon S. Gerig*, Michael J. Dodrill, William E. Pine III

Running head: Habitat selection of humpback chub

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Effective conservation and restoration programs for many native riverine fish communities are highly dependent on regulated river operations developed by water managers. Successfully implementing river flows to meet human needs and minimize ecosystem impacts requires understanding the linkages between hydrology, physical habitat, and fish ecology. In the Colorado River within Grand Canyon, Arizona, altered river conditions following the construction of Glen Canyon Dam have contributed to the decline of humpback chub *Gila cypha*. In 2010 a management experiment was implemented to contrast fluctuating flow management policies with an experimental steady flow. We assessed habitat selection and movement of 30 sub adult humpback chub implanted with acoustic telemetry tags during 2 months of fluctuating flow followed by 2 months of steady flow. We found that telemetered humpback chub used eddies extensively while avoiding runs and were associated with intermediate depths and larger substrates. During both flow treatments, humpback chub exhibited small daily movements of about 100 m per day. No effect of the flow experiment was observed on humpback chub habitat selection or movement. Nearshore habitat use by humpback chub increased during increased turbidity during tributary floods. Larger contrasts in discharge between fluctuating and steady flows may have elicited a stronger response by humpback chub to the steady flow experiment, but our results suggest humpback chub habitat selection and movement is robust to the fluctuating and steady flows observed.
**Introduction**

Habitat protection and restoration have been emphasized as an approach to manage ecosystems versus traditional single species management (Minns et al. 1996; Minns et al. 1999; NRC 2006). Habitat protection and restoration measures are common in the United States and Canada (i.e., Magnuson-Stevens Fisheries Conservation and Management Act, Endangered Species Act, Species at Risk Act) as a primary means to conserve fish and wildlife populations. Many of these efforts however, are not well informed and habitat restoration efforts are rarely evaluated experimentally. Thus, conservation actions are often unable to assess whether habitat use and demographic parameters change following protection or restoration efforts (Van Horne 1983, Quinn and Kwak 2000, Gunn and Sein 2000, Rosenfeld 2003).

Globally, riverine ecosystems have been significantly altered by anthropogenic changes to flow regimes through the widespread construction of dams or other control structures (Dynesius and Nilsson 1994; Poff et al. 1997; Richter et al. 1997; Bunn and Arthington 2002). Large dams often alter natural flow regimes by changing the timing, magnitude, frequency, duration and rate of change of flows within rivers (Poff et al. 1997). These hydrologic changes alter riverine processes including ecosystem productivity, sediment transport, and temperature which may have negative effects on fish populations within riverine ecosystems (Nilsson et al. 2005; Poff et al. 2007). Fish habitat use in rivers is a complex trade-off between numerous abiotic and biotic factors such as thermal environment, flow regime, food availability, competition (Werner and Hall 1979; Butler 1980), and predation risk (Werner et al. 1983; Ahrens et al. 2012).
Anthropogenic alterations to flow have been observed to have widespread effects on the abiotic and biotic factors that structure fish habitat use (Murchie et al. 2008). Widespread habitat loss caused by large dams has been implicated in the decline of native fish in many river systems including the Colorado River basin of the western United States (Tyus and Karp 1991; Minckley 1991). The completion of Glen Canyon Dam in 1963 led to dramatic changes in the hydrology and ecology of the Colorado River ecosystem in Grand Canyon, Arizona (Topping 2003; Gloss and Coggins 2005; Kennedy and Gloss 2005). The pre-dam riverine environment in Grand Canyon was typified by large seasonal changes in discharge, turbidity, and temperature. The post-dam environment has been characterized by elevated base-flow, elimination of seasonal floods, daily fluctuation of discharge for hydropower production, reduction of sediment inputs, and stabilized thermal regime (Topping 2003). Trophic structure shifted from allochthonous to autochthonous production and reduced aquatic invertebrate diversity and abundance (Kennedy and Gloss 2005; Gloss and Coggins 2005; Cross et al. 2011). Native fish populations likely declined from changes to physical habitat and the proliferation of exotic species (e.g. cold-water salmonids; Yard et al. 2011). Restoration efforts on the Colorado River have targeted elements of the historic flow regime to improve physical conditions within the river and benefit native fish populations (Schmidt et al. 1998).

The Glen Canyon Dam Adaptive Management program was implemented in 1996 to address uncertainty in policy actions regarding the effects of Glen Canyon Dam on the Colorado River ecosystem. Conservation of native Colorado River Basin fishes, particularly the endangered humpback chub *Gila cypha* is one of the primary goals of
the program (Melis et al. 2006). As part of the Glen Canyon Adaptive Management Program, a variety of flow experiments have been conducted to assess the physical and biological responses of the Colorado River ecosystem to flow policies (Melis et al. 2006, USFWS 2008, 2011). Two types of flow experiments have been implemented and include experimental steady flow releases and high flow events (Melis 2011). Despite these experimental tests, uncertainty remains regarding how dam operations affect physical and biological resources.

To help address this uncertainty, experimental steady flow releases from Glen Canyon Dam were planned during September and October 2008-2012 in contrast to the modified low fluctuating flow schedule. Modified low fluctuating flow has been the predominant flow policy since 1996 (USFWS 2011). Typical flows from Glen Canyon Dam fluctuate daily between 140 m$^3$/sec to 850 m$^3$/sec. During the experimental steady flow releases discharge from the dam was held constant at 250 m$^3$/sec for 8 weeks each year. Diel fluctuations in discharge have been hypothesized to negatively influence survival of native fish by destabilizing habitats and altering habitat use patterns (USFWS 2008).

In this study, we used the experimental steady flow releases in 2010 to relate dam operations to sub adult humpback chub habitat selection. Humpback chub habitat selection and movement was compared between the steady flow experiment and the modified low fluctuating flow policy. We also evaluated the influence of turbidity on the spatial distribution and habitat use patterns of sub adult humpback chub. Past research on humpback chub habitat use have primarily focused on inferring habitat relationships of adult fish from catch per effort indices and radio telemetry (Kaeding et. al. 1990;
Valdez and Ryel 1997; Converse et al. 1998). However, no studies to date have
quantified sub adult humpback chub habitat use in relation to available habitat to
determine habitat selection patterns and no studies have assessed habitat use during
modified low fluctuating or steady flows. This research will aid managers in evaluating
the importance of habitat features to humpback chub and inform the relationship
between humpback chub habitat selection and Glen Canyon Dam operations.

Methods
Study Site.

A section of the mainstem Colorado River in Grand Canyon, Arizona (RKM 125-
131.2) was sampled during 4 river trips approximately 18 days each in July, August,
September and October of 2010. The modified low fluctuating flow regime (discharges
ranging from 250 m$^3$/sec to 500 m$^3$/sec, Figure 1) was sampled during July and August
and the experimental steady flow regime (constant 250 m$^3$/sec, Figure 1) was sampled
during September and October. Discharge and formazin turbidity units (FTU, hereafter)
were continuously monitored (15-minute intervals) using an acoustic doppler profiler at
USGS gauge 09383100 (RKM 165). The Colorado River Flow Sediment and Stage
model was used to back-calculate the difference in average water travel time between
USGS gauge 0983100 and the study location (Wiele and Griffin 1997). Tributary
flooding during August and September caused spikes in turbidity and flow that were
unrelated to dam operations (Figure 1).

Fish handling and surgery methods.
Thirty two sub adult humpback chub between 180-245 mm TL were surgically implanted with uniquely coded PT-3 Sonotronics® acoustic telemetry tags (Appendix 1) (PT-3: 8-mm diameter, 19-mm length, 1.5 grams, 45-60 day battery life; Sonotronics, Tuscon, AZ, USA). Humpback chub tagged with telemetry tags were captured via hoop netting. Given the size and body shape of humpback chub and guidelines on body size-tag size relationships from the literature, we chose 180-mm TL as the smallest size of humpback chub to implant with an acoustic tag to minimize influence of tagging on survival of fish (Jepsen et al. 2002). All fish were anesthetized in a container with sodium bicarbonate until equilibrium was lost. Fish were transferred to a surgery station where aerated river water was passed continuously over the gills using a gravity fed tank. Tags were implanted by making the smallest possible incision (~8-mm) into the abdominal cavity. The incision was then closed with 2-3 simple interrupted stitches using absorbable suture material and sealed with cyanoacrylate. Each tagged fish was observed for at least 2 hours until the immediate effects of anesthesia and surgery were no longer visible and the fish was able to maintain equilibrium with normal fin movements. Following recovery, fish were released into the same location they were collected. A staggered-entry tagging design was used to increase the temporal resolution of the study (Pollock et al. 1989). All fish were implanted with tags between July-September and tag life was expected to last two months. No fish were implanted during the October trip as viable tags were still active from prior trips.

Tracking telemetered humpback chub.

Fish tracking began 24 hours post-surgery and continued through October or until tag failure. Relocations recorded within 3 days of surgery were censored from all
analyses to allow for a recovery period following tag implantation. Generally, fish were
tracked twice daily during the morning (between 0500 and 0700 hours) and afternoon
(between 1400 and 1600 hours). Tracking efforts were confined to the study area due
to permitting constraints (~ RKM 125-131.2). Tag performance was assessed across a
range of flow conditions and depths during pilot sampling in 2009 and signal attenuation
was observed at distances greater than 100 m. We implemented a systematic tracking
framework to search for telemetered individuals by stopping at fixed locations
approximately 100 m apart. Each location was searched for 3-4 minutes with the boat
moving as slowly as possible. Areas of swift water were floated multiple times to try and
increase likelihood of detecting telemetered fish using these habitats. Telemetry
surveys were not conducted in rapids and turbulent areas because of poor signal
reception and unsafe boating conditions. Following detection of a telemetered fish, the
receiver gain was reduced to increase the precision in locating fish. Once the location
of the fish was determined, the location was entered into a mobile Geographic
Information System (GIS) database and attributes of the location including time, depth,
distance from shore, habitat type and hydraulic type were recorded.

Habitat mapping

Relocations of telemetered humpback chub were related to GIS data layers
consisting of hydraulic type, habitat type, depth and substrate size using both existing
information maintained by USGS Grand Canyon Monitoring and Research Center
(GCMRC) and field measurements. The hydraulic type was mapped in the field and split
into either eddy or run. Eddies refer to areas of recirculating flow that generally occur
downstream of channel constricting debris fans. Runs represent any portion of the river
where the water was traveling downstream. Field measured hydraulic type was digitized in GIS for the study site. Habitat type was delineated using existing GIS imagery and habitat classifications were verified in field. Habitat types were split into six discrete habitat types including backwater, cliff, debris fan, offshore, talus slope and sand (for further characterization see Converse et al. 1998). Backwater habitats are characterized as areas of low flow often associated with sandbars that are partially isolated from the main river channel. Cliff habitats were characterized as shear walls rising vertically and laterally over the river. Debris fans were characterized as shorelines of large cobble and boulder that were transported into the river by tributary flooding. Offshore habitat represented any area that was greater than 15-m from shore. Sand habitats were classified as shorelines of contiguous beach and exposed sand. Talus habitats were classified as shorelines consisting mainly of boulders deposited by rockslides and rock fall. Depth was determined in GIS as surface water elevation minus the elevation of the river bottom for a given discharge. Existing bathymetric and channel coarseness maps were used to classify substrate type into 12 discrete sizes using a modified Wentworth scale. Substrates sizes were grouped into three classes: small, medium and large substrates for the selection ratio analysis. Substrates classified 1-4 represented small substrates ranging from silt to course sand. Substrates classified 5-8 represented small gravel to medium cobble while substrates classified 9-12 represent large substrates including large cobble and boulder. The 12 substrate sizes were used as a continuous variable in the habitat selection models. Habitat availability was determined by generating random locations within the study site using the Arc GIS extension Hawth’s Tools. The number of random locations generated to
quantify habitat availability was equal to the number of fish relocations during each flow
regime. Habitat characteristics were related to available locations in Arc GIS.

Statistical Analysis of Habitat Selection

Following Rosenfeld (2003), habitat selection was inferred as differential use of a
habitat given the availability of the habitat. We used two statistical approaches to
assess habitat selection. First, univariate selection ratios were constructed to compare
individual humpback chub relocation points to available locations within the study area
(Thomas and Taylor 1990; Manly et al. 2002; Rodgers and White 2007). Separate
selection ratios were constructed to evaluate if selection differed between modified low
fluctuating flow and the experimental steady flow release. Second, we developed a
suite of mixed model logistic regressions to investigate the influence of habitat
characteristics on the relative probability of habitat use by sub adult humpback chub.
These two approaches (univariate selection ratios and mixed model logistic regression)
are complimentary methods to study habitat selection each with unique assumptions.
Univariate selection ratios allow for selection to be determined for specific habitat
classes while the mixed model allows a probability of use to be modeled as a function of
habitat characteristics.

Univariate selection ratios were calculated following a type II study design
(Thomas and Taylor 1990; Manly et al. 2002). This design compared the frequency of
habitat use in a discrete habitat class to the availability of the habitat class. Habitat
classes included hydraulic type, habitat type, depth category and substrate category.
Prior to constructing selection ratios, two different chi-square tests were used to test if
humpback chub were uniformly distributed across habitat classes and determine if
habitat selection was occurring. Once selection was established, selection ratios ($W_i$) and Bonferroni adjusted 95% confidence intervals were constructed to determine which habitats were being selected (Manly et al. 2002; Rogers and White 2007). Selection was indicated by values greater than one while avoidance is indicated by values less than one (Rogers and White 2007). Selection ratios equal to one indicated that telemetered humpback chub are not selecting for a particular habitat type (i.e. use is proportional to available habitat). Log-likelihood chi-square tests and selection ratios were calculated using the adehabitat package in Program R (R Development Core Team 2012).

Mixed effects logistic regression models were implemented to compare used versus available locations as functions of habitat attributes for telemetered humpback chub. Nine different a priori models were developed to propose mathematical descriptions of covariates that influence habitat selection. In this analysis, the telemetered fish was considered a random effect while hydraulic type, habitat type, depth, substrate size and flow type (e.g. modified low fluctuating flow versus steady flow release) were fixed effects (Rogers and White 2007). The mixed model framework accommodates the non-normal (binomial) error structure and controls for autocorrelation associated with repeatedly sampling the same fish (Gillies et al. 2006). Model fit was compared using AIC (Burnham and Anderson 2002). The model with the lowest AIC was chosen for interpretation of habitat selection. Mixed effects logistic regression models were implemented using lme4 package in program R. Statistical Analysis of Movement.
To describe the movement patterns of humpback chub, mean daily displacement and extent of movement were calculated for both flow regimes. We calculated mean daily displacement as the linear distance moved between successive relocations during daily morning tracking events and extent of movement as the linear distance between the farthest upstream and downstream relocations. A linear mixed model was used to determine if mean daily displacement varied as a function of flow regime. An individual telemetered humpback chub was considered the random effect while flow regime was the fixed effect (Rodgers and White 2007). Wald’s chi-square statistic was used to assess model significance and a non-parametric bootstrap procedure was used to determine 95% confidence intervals around mean daily displacement. A one-way Kruskal-Wallis test was used to determine if there were differences in extent of movement between flow regimes and a bootstrap procedure (see above) was used to determine confidence intervals. To assess the influence of changing discharge during the fluctuating flow regime on the spatial distribution of telemetered humpback chub a linear mixed-model was used to compare distance to shore to discharge level. To determine the influence of turbidity on spatial distribution we used a linear mixed model to determine if distance from shore varied as a function of turbidity category and a non-parametric bootstrapping procedure was used to assess uncertainty. Three turbidity categories were considered. Low turbidity ranged from 0-30 FTU, medium turbidity levels ranged from 31-300 FTU and high turbidity levels ranged from 301-10,000 FTU. We used a Pierson chi-square test to evaluate if frequency of habitat use changed with increased turbidity.
Results

We recorded 1034 locations from 30 tagged humpback chub during July to October 2010. Thirty two humpback chub were originally implanted with tags but only 30 individuals were known to have remained in the study site. The two humpback chub not relocated were assumed to have emigrated out of the study site or experienced tag failure. These two tags were censored from all analyses. Tagged humpback chub were relocated 344 times during the fluctuating flow regime and 690 times during the experimental steady flow regime. The mean number of relocations for tagged humpback chub was 25 (SD=12.7) and the mean number of days a telemetered fish was observed was 46 (SD=16.2). The mean size of telemetered chub was 199 mm (SD=19.2) and ranged between 180 mm to 245 mm (Appendix 1).

Habitat Selection.

Habitat selection was assessed for 14 sub adult humpback chub during the fluctuating flow regime and for 25 sub adult humpback chub during the steady flow experiment. During both flow regimes locations used by humpback chub differed significantly (P-value <0.05) from availability for all habitat characteristics except for substrate category during the fluctuating flow regime (Table 1). The largest selection ratios were for hydraulic type, habitat type, and depth category (Figure 2). Humpback chub strongly selected eddy hydraulic types and these areas were used in proportions more than three times their availability. Runs were avoided during both flow regimes. Habitat types including cliff and debris fan shorelines were positively selected for during both flow regimes. Selection for backwater habitat was highly uncertain during both the fluctuating and stable flow regimes due to the small number of observations in this
Due to the limited availability of backwater habitat the high selection ratios for this habitat may be inflated. Fish demonstrated no selection or avoidance for talus during fluctuating flows while positively selecting this habitat type during steady flows. Offshore and sand habitats were avoided during both flow regimes. Telemetered humpback chub selected intermediate depths of 4-6 m while generally avoiding shallow (<2 m) and deep areas (> 10 m) during both flow regimes (Figure 2). Tagged humpback chub showed no selection or avoidance of substrate class during both flows with the exception of positive selection of the medium sized substrate class during the steady flow regime (Figure 2).

**Mixed Model Logistic Regression.**

The most highly supported mixed model describing humpback chub habitat selection included hydraulic type, depth, substrate size and an interaction between hydraulic type and substrate size (Table 2). These variables best explained the probability of habitat selection by humpback chub. Hydraulic type was the strongest predictor of habitat selection causing the largest improvement in model fit (Table 2). Similar to the selection ratio analysis, eddy hydraulic types were used in much greater proportion to their availability and runs were avoided (Table 3). Telemetered humpback chub used shallower depths and larger substrate than would be expected based availability. There was a significant interaction effect between substrate category and hydraulic type. This interaction indicated that humpback chub used a wide variety of substrate categories in eddies while being negatively associated with small substrates and positively associated with larger substrates in runs. Including a parameter that represented the contrasting flow releases did not improve model fit suggesting that
habitat selection did not change between fluctuating and steady flow regimes (Table 2, delta AIC = 2 between competing models).

Movement.

Mean daily linear movements of telemetered humpback chub were not significantly different between the fluctuating and experimental steady flow release ($\chi^2 = 0.16, P = 0.6867$). During the fluctuating flow regime, tagged humpback chub moved a mean distance of 92 m per day (95% confidence interval, 67-123 m; Figure 3) while during the steady flow regime telemetered chub moved a mean distance of 106 m per day (95% confidence interval, 91-123 m; Figure 5). In both flow regimes, long distance daily movements (> 500 m) were rare. Significant differences in extent of movement were not observed between the fluctuating and steady flow regime for telemetered humpback chub ($\chi^2 = 0.14, P = 0.7015$). The mean extent of tagged fish during fluctuating flow was 414 m (95% confidence interval, 258-595 m; Figure 3) and during the steady flow experiment mean extent of movement was 515 m (95% confidence interval, 333-743 m; Figure 3). No relationship was found between humpback chub distance from shore and discharge level ($\chi^2 = 1.56, P = 0.20$) suggesting that humpback chub do not alter spatial distribution in response to changing discharge under the discharge levels observed.

Turbidity.

A significant relationship ($\chi^2 = 77.87, P < 0.01$) was found between distance from shore and turbidity level. During low turbidity conditions humpback chub were located further offshore than during high turbidity conditions (Figure 3). Similarly, during this study the frequency of nearshore habitat use for all nearshore habitat types increased
with higher turbidity levels ($\chi^2=63$, df=10, P<0.01). This pattern was strongest in backwater, cliff and debris fan habitat types (Table 4). The frequency of use of backwater habitat changed with increased turbidity. No fish were relocated in backwater habitat during low turbidity and fish were relocated more than 50 times during increased turbidity conditions.

**Discussion**

We found that sub adult humpback chub habitat selection and movement patterns did not differ between fluctuating and the experimental steady flow treatments. Our results show that telemetered humpback chub exhibit strong selection for eddy hydraulic types across river flows. Nearshore habitats including debris fan and cliff faces located within eddies were also positively selected during both flow treatments. Prior research has demonstrated that adult humpback chub use eddy complexes extensively and that the distribution of humpback chub in Grand Canyon may be related in part to the presence of large eddy complexes (Valdez and Ryel 1995). Kaeding et al. (1990) further postulated that adult humpback chub rely on large channel obstructions such as debris fans to create velocity refuges via eddy complexes. In Grand Canyon, eddies generally occur below rapids caused by channel constricting debris fans. Eddies create areas of recirculating flow adjacent to the main current and increase material retention time as a function of slower average water velocities when compared to runs (Schmidt 1990). Our results for sub adult humpback chub concur with previous research on adult fish and suggest that the mechanisms causing strong selection of eddy habitats by humpback chub are likely related to food availability and reduced energetic expenditure (Valdez et al. 2001). Residing in depositional environments
adjacent to turbulent main channel flow may allow humpback chub to maintain position in lower velocity water while making foraging attempts at entrained organic matter and invertebrates in the water column. Similar patterns have been observed for cyprinids in small stream systems in the southeastern and southwestern United States (Rinne 1991, Freeman and Grossman 1993).

We observed that sub adult humpback chub make small daily movements and exhibit a restricted distribution. Most movements of tagged humpback chub were made within large eddy complexes. However, movements between eddy complexes and from runs to eddies were observed. Previous studies with adult humpback chub using telemetry and passive tags have shown that over both short and long temporal (e.g. weeks to several years) scales humpback chub exhibit strong patterns of spatial fidelity (Valdez and Ryel 1995; Valdez et al. 2001; Paukert et al. 2006). Seasonal movements of adult humpback chub in Grand Canyon are characterized by potadromous spawning migrations between the mainstem Colorado River and the Little Colorado River between March and May (Gorman and Stone 1999, Coggins et al. 2006). Our sampling period did not overlap with the timing of this migration and no evidence of movement between the mainstem and Little Colorado River was documented for telemetered humpback chub considered here. Long distance movements in excess of 50 km by humpback chub have been observed through long-term tagging studies (Paukert et al. 2006). However, given the limited movements observed in this study it appears that long distance dispersal may be rare during the late summer and fall. This result is important in planning mark-recapture studies where knowledge of limited movement is important
in assessing whether or not the population is closed to immigration or emigration during
the study time period (Gwinn et al. 2011).

Large recirculating eddies are likely an important physical habitat feature for sub
adult humpback chub in the Colorado River in Grand Canyon based on the results from
this study. Humpback chub that occupy the mainstem Colorado River in close proximity
to the Little Colorado River may be able to meet their requirements for foraging and
resting within this limited spatial extent. Future research should quantify fitness metrics
such as growth or survival that may differ between habitat types to further identify
habitat requirements. Our inferences were restricted to only a few kilometers of habitat
in the Colorado River where the largest aggregation of humpback chub are found
(Coggins et al. 2006). Future efforts could assess habitat selection in other reaches of
the Colorado River, particularly in areas where humpback chub population density is
much lower such as western Grand Canyon or Westwater Canyon-Black Rocks in the
upper Colorado River. This information may help to determine important physical
factors that affect the distribution of humpback chub and inform management actions in
Grand Canyon and the Upper Colorado River Basin.

Overriding turbidity conditions appeared to have a stronger influence on the
spatial distribution of humpback chub than the experimental steady flow regime. The
proportion of relocations in nearshore habitats increased concomitantly with turbidity
levels and the spatial distribution of humpback chub shifted closer to shore. Turbidity
has been identified as factor of overriding importance for humpback chub catch rates in
hoop-nets within the Little Colorado River (Stone 2010) and is known to cause
behavioral responses in many species (Gregory 1993; Sweka and Hartman 2001). An
upper turbidity threshold of 545 nephalometric turbidity units (NTU) has been identified in the Little Colorado River for humpback chub beyond which the visual capabilities of fish are overwhelmed, leading to behavior changes, such as decreased reliance on cover (Stone 2010). This study supports the view that turbidity is an important factor influencing behavior and spatial distribution of humpback chub within the mainstem Colorado River.

We did not relocate any telemetered fish in backwater habitats which are commonly considered important habitat for juvenile humpback chub (Hoffnagle 1996, Hoffnagle 2000) when turbidity conditions were less than 30 FTU. However, as turbidity increased, fish were relocated in backwater habitats over 50 times. The shift in humpback chub spatial distribution may be related to behavioral responses to light, predation risk, or foraging behavior. Under low turbidity conditions humpback chub likely forage offshore on material entrained in eddy currents. As turbidity increases humpback chub increasingly use nearshore habitats and may become actively or opportunistically piscivorous (Valdez and Ryel 1995; Stone and Gorman 2006).

Backwater seine surveys and short term tethering experiments which occurred concurrently to the telemetry study found that the frequency of large fish, including sub adult and adult humpback chub captured in backwaters increased with turbidity and that under moderately turbid conditions (mean 146; range 27 – 818 FTU) predation risk in tethering trials was highest (Dodrill 2012). Additionally, on two occasions during turbid conditions during the tethering experiments, humpback chub were observed engulfing tethered fish, apparently foraging. (M. Dodrill, personal observation). Prior research in Grand Canyon suggests that the abundance of small bodied fish increased with turbidity
as a result of dispersal from tributaries during floods (Yard et al. 2011).

Correspondingly, these results suggest that humpback chub shift their habitat use patterns during turbidity fluxes to potentially forage on fish in nearshore habitats such as backwaters. Additional research is needed to determine the possible response of humpback chub and other fish species to the altered turbidity and sediment regimes currently being considered as management alternatives for the Colorado River in Grand Canyon (Randle et al. 2007).

Management Implications.

A common area of research in managing regulated rivers is understanding what physical habitat features are used and required by the species of management concern. In the Grand Canyon reach of the Colorado River, this uncertainty has persisted through a lack of knowledge of what physical habitat features are used by juvenile and sub adult humpback chub. We found that sub adult humpback chub exhibited habitat selection and movement patterns that were robust to discharge fluctuations, possibly due to the small magnitude of difference between the modified low fluctuating flow and the stable flow experiment. Despite differences in discharge observed during 2010 as part of the flow experiment, the ranges of flows observed in this study were within the operational constraints of the extant modified low fluctuating flow conditions. If the contrasts in flow regimes had been larger, either higher or lower flows than the modified low fluctuating flow policy, greater humpback chub responses to the steady flow experiment may have been observed. Our study reach was primarily comprised of large eddy complexes adjacent to complex high angle shoreline habitat. These habitats are relatively invariant to discharge fluctuations, likely contributing to telemetered humpback chub habitat.
selection patterns being robust to changes in discharge (Korman et al. 2004). In contrast, low angle habitats such as those in the Lee’s Ferry reach of the Colorado River, are more susceptible to changes in stage than our study reach (Korman and Campana 2009). Second, smaller size classes (~ 180-mm TL used here) of humpback chub may be more reliant on nearshore habitats (Stone and Gorman 2006) and more affected by daily fluctuations in river stage. Habitat selection for these smaller size classes of fish is assessed elsewhere (Dodrill 2012).

The Colorado River in Grand Canyon is one of the most intensively studied riverine ecosystems in the world, yet fish population responses to management actions in this system are usually much smaller than anticipated, or occasionally even counterintuitive, to the expected response (Pine et al. 2009). This may be partly due to our unrealistic expectations of how rapidly resources may respond to a management “treatment” or the scale of the treatments applied in this system. As an example, some aspects of the riverine environment such as temperature or habitat availability may respond almost immediately to flow treatments. However, biological responses such as fish changing their habitat selection patterns or colonizing new habitats may not happen or happen over longer time scales than the flow treatment was made. We suggest that substantial learning can take place when managers contrast observed vs. expected responses to management actions and seek to understand why the system did, or did not, respond as anticipated. This type of iterative assessment should help managers develop more effective policies to aid in informing future management actions by more clearly identifying our shortcomings and successes in predicting ecosystem responses to river regulations.
Acknowledgements

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fish habitat and rehabilitation. American Fisheries Society, Symposium 22, Bethesda, Maryland.


Table 1. Chi-square statistics testing (1) the distribution and (2) selection of habitat characteristics of humpback chub during contrasting flow releases.

<table>
<thead>
<tr>
<th>Habitat Characteristic</th>
<th>Distribution</th>
<th>Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flow Type</td>
<td>χ²</td>
</tr>
<tr>
<td>Habitat Type</td>
<td>Fluctuating</td>
<td>83.87</td>
</tr>
<tr>
<td></td>
<td>Steady</td>
<td>164.07</td>
</tr>
<tr>
<td>Hydraulic Type</td>
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<tr>
<td></td>
<td>Steady</td>
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<tr>
<td>Depth Class</td>
<td>Fluctuating</td>
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<tr>
<td></td>
<td>Steady</td>
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<tr>
<td>Substrate Class</td>
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<tr>
<td></td>
<td>Steady</td>
<td>64.27</td>
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Table 2. AIC ranking of mixed model logistic regression models used to determine habitat selection of telemetered, subadult humpback chub.

<table>
<thead>
<tr>
<th>Model</th>
<th>LL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>K&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Depth+ Substrate Size+ Hydraulic Type+ (Substrate Size*Hydraulic Type)</td>
<td>-1073</td>
<td>2157</td>
<td>0</td>
<td>5</td>
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<td>Depth+ Substrate Size+ Hydraulic Type+ Flow Release+(Substrate Size*Hydraulic Type)</td>
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<td>2159</td>
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<td>5</td>
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<tr>
<td>Depth+ Substrate Size+ Hydraulic Type+ (Depth*Hydraulic Type)</td>
<td>-1076</td>
<td>2163</td>
<td>6</td>
<td>5</td>
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<tr>
<td>Depth+ Substrate Size+ Hydraulic Type+ Flow Release+ (Depth*Hydraulic Type)</td>
<td>-1074</td>
<td>2165</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Depth+Depth²+Substrate Size+Hydraulic Type</td>
<td>-1080</td>
<td>2171</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Depth+Substrate Size +Hydraulic Type</td>
<td>-1081</td>
<td>2172</td>
<td>15</td>
<td>4</td>
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<td>71</td>
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<td>2607</td>
<td>450</td>
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<td>Depth+Substrate Size</td>
<td>-1364</td>
<td>2736</td>
<td>579</td>
<td>3</td>
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<sup>a</sup> = Log Likelihood;  <sup>b</sup> = # of parameters
Table 3. Coefficient estimates from top AIC ranked mixed model logistic regression for telemetered sub adult humpback chub during contrasting flow releases.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>z value</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
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<td>Depth</td>
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<td>Substrate</td>
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</tr>
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<td>Substrate * Hydraulic Type</td>
<td>-0.27</td>
<td>0.070</td>
<td>-4.10</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Table 4. Frequency of habitat use during contrasting turbidity conditions for telemetered sub adult humpback chub. Low turbidity ranged between 0-30 FTU, medium turbidity was between 31-300 FTU, and high turbidity ranged from 301-10,000 FTU.

<table>
<thead>
<tr>
<th></th>
<th>Backwater</th>
<th>Cliff</th>
<th>Debris Fan</th>
<th>Offshore</th>
<th>Sand</th>
<th>Talus</th>
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<tr>
<td>Low</td>
<td>0</td>
<td>13</td>
<td>17</td>
<td>90</td>
<td>6</td>
<td>41</td>
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<tr>
<td>Medium</td>
<td>16</td>
<td>57</td>
<td>65</td>
<td>189</td>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>High</td>
<td>35</td>
<td>101</td>
<td>80</td>
<td>169</td>
<td>8</td>
<td>62</td>
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</table>
Figure 1. Map of study location in the Colorado River in Grand Canyon
Figure 2. Mean, maximum and minimum daily discharge during modified low fluctuating flow and the experimental steady flow releases and daily turbidity measurements from July to October 2010 in the Colorado River in Grand Canyon.
Figure 3. Selection Ratios with 95% Bonferroni confidence intervals of (A.) habitat type, (B.) hydraulic type, (C.) depth category (m) and (D.) substrate category. Black data points represent modified low fluctuating flow while gray data points represent steady flow releases.
Figure 4. Movement and spatial distribution of telemetered humpback chub during contrasting flow releases and turbidity levels. (A.) Daily movement, (B.) spatial extent of telemetered humpback chub during the fluctuating and experimental steady flow regime and (C.) observed distance from shore of humpback chub during low, medium and high turbidity levels.
Supplemental Information. Biological attributes collected from sub adult humpback chub implanted with acoustic telemetry transmitters.

<table>
<thead>
<tr>
<th>Trip</th>
<th>Length (mm)</th>
<th>Weight (g)</th>
<th>ID</th>
<th>Date Tagged</th>
<th>Last Contact</th>
<th>Tracking Duration (d)</th>
</tr>
</thead>
<tbody>
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<td>1</td>
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<td>52.73</td>
<td>711050</td>
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<td>9/15/2010</td>
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<tr>
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<td>8/12/2010</td>
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<td>8/12/2010</td>
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<td>8/18/2010</td>
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<tr>
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SITE OCCUPANCY OF HUMBACK CHUB IN THE COLORADO RIVER IN GRAND CANYON, AZ

Brandon S. Gerig and William E. Pine III

Abstract

We assessed habitat use of humpback chub *Gila cypha* using site occupancy models in the Colorado River near Grand Canyon, Arizona. We examined whether site occupancy changed for different size classes of humpback chub during two flow regimes, the extant peaking hydropower flows from Glen Canyon Dam and a contrasting approximately 60 day period of steady flows. Using this approach, we evaluated the importance of time varying, size specific, site specific, and pass specific covariates on patterns of site occupancy while accounting for changes in detectability. We found occupancy probabilities were consistently high and there was no marked change in occupancy state between the extant hydropumping and steady flow treatments. Because estimates of occupancy probabilities were high, we were unable to find relationships between habitat covariates and humpback occurrence – basically because all sites assessed were equally likely to be occupied possibly related to humpback chub abundance in this river reach. We did find that detection probabilities exhibited considerable heterogeneity reflecting differences among sampling trip, humpback chub size class, and habitat covariates. Future research should examine humpback chub site occupancy in a Colorado River reach where habitat types are more stage variant and humpback chub densities are lower than our study site near the Little Colorado River.
Introduction

Fish habitat use in rivers is a complex trade-off between numerous abiotic and biotic factors such as thermal environment, flow regime, food availability, competition (Werner and Hall 1979; Butler 1980), predation risk (Werner et al. 1983; Walters and Martell 2004), and shifts between foraging and resting areas. Habitat use and selection often varies with life-stage for many organisms and ontogenetic habitat shifts are commonly adopted by fish to utilize specific spawning, nursery and foraging areas (Werner et al. 1988; Walters and Juanes 1993; Faush et al. 2002; Walters and Martell 2004). Understanding the temporal and spatial patterns of habitat use is a critical need in ecology (Johnson 1980; Morrison and Marcot 2006).

Observations of animal habitat use are often used to inform decisions about habitat requirements thus informing management policies designed to protect specific habitat types and species (Van Horne 1983). Increasingly, efforts to manage aquatic resources have emphasized habitat protection as an approach to manage ecosystems versus traditional approaches of managing single species (Minns et al. 1996; Minns et al. 1999). Habitat protection and restoration measures have been mandated in the United States (Magnuson-Stevens Fisheries Conservation and Management Act, Endangered Species Act) as a primary means to conserve fish populations from decline. However, many of these efforts are not well informed and habitat mitigation and restoration efforts are rarely assessed experimentally thus they often fail to assess whether habitat use and population vital rates change following protection or restoration efforts (Van Horne 1983, Quinn and Kwak 2000, Gunn and Sein 2000, Rosenfeld 2003).

The completion of Glen Canyon Dam in 1963 led to dramatic changes in the hydrology and ecology of the Colorado River ecosystem in Grand Canyon (Topping 2003; Gloss and Coggins 2005; Kennedy and Gloss 2005). The Glen Canyon Dam Adaptive Management program was implemented in 1996 to address uncertainty in policy actions on the operation of Glen Canyon Dam on resources within the Colorado River ecosystem with the conservation of native Colorado River Basin fishes as one of the goals of the program (Melis et al. 2006). In 2008 an adaptive management experiment was implemented to assess fish population responses to a series of planned steady flow releases from Glen Canyon Dam. Glen Canyon
Dam is typically operated as a peak hydropower facility under the modified low fluctuating flow regime in which the dam is peaked daily between 140 (m$^3$/sec) to 850 (m$^3$/sec) to produce hydroelectric power. Dam operations during September and October from 2009-2011 were scheduled to operate under a steady flow regime allowing humpback chub populations to be monitored during contrasting flow regimes. It was hypothesized that diel fluctuations in discharge could influence survival of native fish by destabilizing nearshore habitats that are thought to be important to juvenile humpback chub. Further, it was hypothesized that the steady flow regimes would raise temperature of nearshore areas and improve humpback chub growth rates (see Pine et al. *in review* for additional background and details).

In this river reach, a variety of long-term fish monitoring programs have tracked status and trends in native fish populations (Coggins et al. 2006) but most work on habitat use and requirements has focused on the Little Colorado River (Gorman and Stone 1999; Stone and Gorman 2005). There is limited published information on patterns of habitat use in humpback chub in the mainstem Colorado River (see Converse et al. 1998; Gerig et al. *in-review*) and in particular our understanding of the role dam operations play in structuring habitat use patterns is poorly understood.

The goal of this study is to assess habitat use of humpback chub using site occupancy models (MacKenzie et al. 2006) in the Colorado River in Grand Canyon during two contrasting flow regimes. Our intent is to evaluate the impact of a steady flow regime from Glen Canyon Dam compared to extant peaking hydropower operations on humpback chub. This approach allows the estimation of un-biased habitat use or occupancy probabilities by explicitly accounting for heterogeneity in detection. Using this approach, we evaluated the importance of time varying, size specific, site specific and pass specific covariates on patterns of site occupancy while accounting for changes in detectability. This information on habitat use of humpback chub will help guide the management of the Colorado River in Grand Canyon by providing managers with insight into the influence of flow regime on habitat use for a species of significant management interest.
Methods

Study Area

This study was conducted within the Little Colorado River inflow area of the Colorado River in Grand Canyon (Figure 1). Our study area was approximately 4.2 km in total length and located between Heart Island (RM 125 RKM) and Lava Chuar Rapid (RKM 131.2). The study area was subdivided into three reaches approximately 1500 m in length (3,000 m total shoreline). Each of the study reaches was separated by small (~1.5-m height) rapids. Within each reach the shoreline was further broken up into adjacent 50-m nearshore sites using aerial photographs in a geographic information system (Figure 2). We sampled each reach during 12 river surveys in July, August, September, and October of 2009 through 2011 as part of the Nearshore Ecology Project.

Discharge Patterns

Discharge patterns were continuously monitored at 15-min intervals remotely at USGS gauge 09383100 (RKM 165). The Colorado River Flow Sediment and Stage model was used to back-calculate the difference in average water travel time between USGS gauge 0983100 and the study location (RKM 125-131.2) using an unsteady flow model (Wiele and Griffin 1997). As part of this study, July and August surveys during 2009 and 2010 sampling surveys occurred during the modified low fluctuating flow regime (MLFF, diel discharges of 250 to 500 m$^3$ sec in 2009; 300 to 500 m$^3$ sec in 2010), and September and October sampling surveys were sampled during the low steady flow regime (constant flows of 250 m$^3$ sec in 2009; 300 m$^3$ sec in 2010; Figures 3 and 4). Due to increases in upper basin water inputs to Lake Powell in 2011 and record low reservoir levels in Lake Mead, discharge patterns in 2011 from GCD were a high steady flow regime (HSF) during July and August (constant flows of 715 m$^3$ sec) and a medium steady flow regime (MSF) during September and October (constant flows of 460 m$^3$ sec; Figure 5). Extensive tributary flooding during August and September 2010 caused increases in discharge that were unrelated to dam operations (Figure 6). Daily turbidity measurements were continuously monitored in 15-minute intervals remotely from an acoustic Doppler profiler at USGS gauge 09383100 (RKM 165). An unsteady flow model was used to back calculate water
travel time to the study area (Wiele and Griffin 1997). Spikes in turbidity were caused by
tributary floods.

**Sampling Procedures**

Hoopnets and boat electrofishing were used to sample humpback chub during this study
to evaluate patterns of site occupancy. Eighty hoopnets were set within designated sites in the
first and second sampling reach. The hoopnet sites were fished during each of the four yearly
sampling surveys during 2009 to 2011. Hoopnets were fished for 24 hour passes over 12
consecutive nights during each sampling survey. Boat electrofishing was conducted in sampling
reaches one, two and three. The effective sampling area of hoopnets boat electrofishing was
estimated to be a 50-m site within 4-m of shore. Sampling reach one had 58 sites, sampling
reach two had 49 sites and sampling reach three had 52 sites. This sampling design allowed for
all shoreline areas excluding rapids in our sampling area to be sampled completely. During
2009, each site within a reach was repeatedly sampled three times over a five day period for
reaches one and two (48 hours between passes) and over successive nights in reach 3 (24 hours
between passes). During 2010 and 2011, each site within a reach was repeatedly sampled
three times over successive nights (24 hours between passes). Electrofishing surveys were
conducted using two-6 m Osprey boats outfitted with either a Smith-Root Type 12b or Coffelt
CPS system powered by a Honda 4500 watt generator to apply a complex pattern of pulsed DC
(Speas et al. 2001, Korman et al. 2009). During each electrofishing pass, nearshore sites were
shocked slowly (about 10 m$^{-1}$ sec). All electrofishing sampling began at dark between 1800
hours and 2000 hours depending on season. Surveys were generally finished by 0400 hours.
All fish captured during hoopnet and electrofishing sampling were processed and returned to
the site from which they were captured following standard Grand Canyon fish cooperator
sampling protocols (Persons et al. 2011).

Catch data for humpback chub were stratified by size class and converted to a binary
encounter history of presence-absence observations. Hoopnet catches by pass were stratified
into three size classes: 40-80 mm, 81-150 mm, and 150-250 mm. Electrofishing catches by pass
were stratified into 40-80 mm and 81-150 mm size classes. Size classes were determined by
visually analyzing length-frequency histograms. Humpback chub greater than 150 mm from
electrofishing samples and greater than 250 mm in hoopnet surveys were excluded from occupancy analyses because of infrequent captures.

### Habitat Classification and Covariates

A suite of site specific categorical and continuous habitat covariates were used for modeling humpback chub detection and occupancy probabilities (Table 1). Categorical habitat covariates used included habitat type and hydraulic type. Continuous habitat covariates included substrate proportion, mean depth and mean site depth². Habitat type in each site was classified in field into four discrete habitat types: cliff, debris fan, talus slope and sandbar following Converse (et al. 1998). Cliff habitats were characterized as shear walls rising vertically and laterally over the river. Debris fans were characterized as shorelines of large cobble and boulder that were transported into the river corridor by tributaries during flooding. Sandbar habitats were classified as shorelines of contiguous beach and exposed sand. Talus habitats were classified as shorelines consisting mainly of boulders deposited by rockslides and rockfall. The hydraulic type of a site was mapped in the field and split into two discrete types: eddy flow and downstream flow. Eddies represent areas of recirculating flow that generally occur downstream of channel constricting debris fans. The downstream category represents any portion of the river where the water is traveling downstream. Substrate classes were grouped into three classes: small, medium and large substrates using a modified Wentworth scale. In GIS, a 4-m (electrofishing sites) and 10-m (hoopnet sites) buffer was applied to the wetted river perimeter and the proportion of each substrate category in the site was calculated. Substrates classified as small ranged from silt to course sand. Substrates classified as medium represented small gravel to small cobble. Substrates classified as large ranged in size from large cobble to boulder. A bathymetry map of the study reach was used to determine the mean elevation of each hoopnet and electrofishing site along the shoreline perimeter. Similar to the substrate classification a 4-m and 10-m buffer were applied to electrofishing and hoopnet sites. Depth was determined as the average shoreline elevation minus the average elevation of the river bottom within each site.
The estimation of occupancy and detection probabilities required the use of presence absence data from repeated hoopnet and electrofishing passes. Multi-season occupancy models estimated probabilities of site occupancy and detection probability using the encounter histories \( h_i \) within a given site for a given survey (MacKenzie et al. 2006, Donovan and Hines 2007). As an example, an observed \( h_i = 010 \) represents a site that was sampled for 3 passes in a survey. However, because of incomplete detection, the species was observed only during the second pass (even though the species was present all three sampling times). The probability of \( h_i = 010 \) is thus:

\[
P(h_i) = \Psi(1 - p_1)(p_2)(1 - p_3)
\]

where \( \Psi \) is the probability of site occupancy, \( p_i \) is the probability that a species is detected in pass \( i \) and \( 1-p_i \) is equal to the probability that the species is not detected during pass \( i \). An observed detection history, \( h_i = 000 \) indicates that during each of the 3 passes the species of interest was not detected. Assuming that detection probability is less than one this detection history can arise two ways, either the species is truly absent or the species is occupying the site yet, is not detected. The probability of \( h_i = 000 \) is:

\[
p(h_i) = \Psi(1 - p_1)(1 - p_2)(1 - p_3)(1 - \Psi)
\]

where \( \Psi(1-p_1)(1-p_2)(1-p_3) \) is the probability that the species of interest was present at the site but undetected and \( (1-\Psi) \) represents the probability that the species of interest does not occupy the site of interest. Detection \( (p) \) and occupancy \( (\Psi) \) were estimated by maximizing the following model likelihood:

\[
L(p, \Psi|h_i) = \prod \text{Pr}(h_i),
\]

which represents the product of each encounter histories \( (h_i) \) probability (MacKenzie et al. 2002, MacKenzie et al. 2006, Donovan and Hines 2007). A logit link function was used to model the effect of covariates on occupancy and detection probabilities. The logit link function is specified as:

\[
\ln[\theta / (1 - \theta)] = \beta_0 + \beta_1 x_{i1} + \cdots + \beta_j x_{ij},
\]

where \( \theta \) is the parameter of interest (occupancy or detection), \( \beta_j \) is the effect of the given covariate \( x_{ij} \) and \( \beta_0 \) is the intercept of the model.
Assumptions of multi-season occupancy models included: (1) Detectability and occupancy probability of humpback chub is constant among sites or heterogeneity in detection and occupancy can be modeled completely with the inclusion of covariates, (2) detection of humpback chub are independent at each site, and (3) sites are closed to changes in occupancy by humpback chub during the course of a sampling survey (season). We further assumed that assumption three could be relaxed if movement in and out of a site occurred randomly (MacKenzie et al. 2006). When random localized movement occurs it is appropriate to restrict our interpretation of occupancy models to the proportion of sites used rather than the proportion of sites occupied (MacKenzie et al. 2006).

Model Structure (p and Ψ)

_A priori_ models were developed to propose mathematical descriptions of factors that influenced site occupancy and detection probability of humpback chub (Table 1). Following the recommendation of MacKenzie et al. (2006), models were fit for detection probability first and the best model for detection was selected to model site occupancy. Covariates included in models were presumed to be biologically important to occupancy and detection probabilities. Time varying, size specific, site specific and pass specific covariates were considered. Time varying covariates included survey (sampling trip, 1-12), flow regime (e.g. MLFF, Modified Low Fluctuating Flow; LSF, Low Steady Flow; MSF, Medium Steady Flow; HSF, High Steady Flow), year (e.g. 2009, 2010, 2011) and year + flow regime (e.g. unique parameter for each year-flow regime combination). Size specific covariates included humpback chub size class. Site specific covariates included sampling reach (electrofishing only), mean site depth, substrate proportion (e.g. small, medium, large), site habitat type and site hydraulic type. Mean site depth$^2$ was also considered to determine if site occupancy and detection exhibited a non-linear response to this covariate. Turbidity was considered a pass specific covariate and was only modeled for detection probability (Table 1).

Competing detection and occupancy models were compared using Akaike’s Information Criteria (AIC; Burnham and Anderson 2002). This approach ranks competing models by comparing maximized log-likelihoods while applying a penalty for the number of model parameters. The difference in AIC values between models ($Δ$AIC) was used to determine the top
model that was parsimonious, biologically reasonable and with low AIC. Model weights \( w_i \) were calculated to determine the support each model received given the data. Because of model uncertainty only models with AIC \( w_i > 0.05 \) were used to draw inference. All occupancy models were fit using Program Presence 3.1 (Hines 2011).

**Results**

**Catch Composition**

Eighty hoopnet sites were sampled over the course of 12 sampling surveys in 2009 to 2011. Because of variations in hoopnet site location and increased number of hoopnet sites in 2010 and 2011 only 57 sites were included in the analyses of hoopnet samples to maintain consistency in sites sampled. A total of twelve fish species were collected in hoopnet surveys while 15 species were sampled during electrofishing surveys. During hoopnet surveys humpback chub (HBC) were the numerically dominate catch in 2009-2011 accounting for 74%, 72.5% and 76% of the total catch (Table 2). A total of 158 electrofishing sites were sampled a total of 36 times over 12 surveys in 2009 to 2011. Humpback chub (HBC) accounted for 16% and 10% of the catch in 2009 and 2010 while increasing to 24% of the total catch in 2011 (Table 3).

**Detection Probabilities**

**Hoopnets**

A total of fourteen models were constructed to assess heterogeneity in detection across a range of physical, temporal and biological attributes (Table 1). The best fitting model estimated a range of detection probabilities between 0.05-0.33 and included sampling survey, fish size, and a quadratic form depth (including mean depth + mean depth squared in model statement) as covariates (Figure 7, Table 4). Detection probabilities were lowest for humpback chub 150-250 mm and highest for chub 40-80 mm and 81-150 mm. Humpback chub 150-250 mm were detected significantly less than chub between 40-80 mm and 81-150 mm across all sampling surveys with the exception of October of 2009 (Figure 7). The best fitting model also indicated that the probability of detection was highest at intermediate depths (Figure 8). Detection models that included other habitat covariates were not supported by the data (Table 4). Models that did not include sampling survey and fish size as covariates also performed
poorly (Table 4). There was no support for models that modeled heterogeneity of detection as a function of year of sampling or flow regime (Table 4).

**Electrofishing**

A total of sixteen models were constructed to model heterogeneity in detection probability of humpback chub captured during electrofishing surveys (Table 5). The top detection model included sampling survey, sampling reach, humpback chub size class and substrate heterogeneity (proportion of medium sized substrate within the site sampled) as important covariates (Table 5). Detection probability estimates for this model ranged by time and fish size from the highest values in September of 2011 (range=0.18-0.54) to lowest in October of 2009 (range=0.05-0.22) (Figures 9-10). Detection probabilities for humpback chub between 40-80 mm captured during electrofishing surveys were always higher than detection probabilities for fish between 81-150 mm across all sampling surveys and sampling reaches. Detection probabilities for both 40-80mm and 81-150 mm humpback chub exhibited a monotonic decline from sampling reach one to reach three in 2009 through 2011 (Figures 9-11). The best fitting model also indicated that there was a positive relationship between the probability of detection and substrate heterogeneity (proportion of medium substrate within a habitat sampling unit) (Figure 12). The second best fitting model, ranked by AIC was parameterized similarly to the top model except that a negative relationship between detection probability and substrate homogeneity (proportion small substrate within a site) was observed (Figure 13). Detection models that included other habitat covariates (Tables 4-5) were not supported by the data. Models that did not include survey, reach and size as covariates fit the data poorly (Tables 4-5). There was no support for models that modeled heterogeneity of detection as a function of year of sampling or flow regime.

**Occupancy Probabilities**

**Hoopnets**

A total of thirteen models were constructed to model heterogeneity in occupancy (habitat use) probability of humpback chub captured in hoopnets as a function of habitat, temporal and biological attributes. The best fitting model indicated that occupancy probability varied as a function of survey of sampling and size category of humpback chub (Table 6).
Estimated occupancy probabilities were relatively consistent among surveys and size class with a mean occupancy probability of 0.80 (Figure 14). During all surveys chub 151-250 mm exhibited the lowest occupancy probability but this result was not statistically significant. The inclusion of habitat covariates to explain occupancy did not result in a significant improvement in model fit or significant habitat relationships (Table 6).

Electrofishing

A total of eighteen models were constructed to model occupancy (use) probability of humpback chub observed in electrofishing surveys. The model best supported by the data indicated that occupancy probability varied as a function of year, flow regime and size category. Overall occupancy probabilities ranged from 0.72 -0.90 for chub 40-80 mm and 0.28-0.59 for fish 81-150 mm (Figure 15). Occupancy probabilities for humpback 40-80 mm and 81-150 mm declined slightly through time (Figure 15). Eight additional models were within a $\Delta$ AIC of 5 of the top model (Table 7). Each of these models were parameterized identically to the top model except that they each had a habitat covariate included. The inclusion of habitat covariates in occupancy models did not significantly improve model fit (Table 7).

Discussion

During this study, occupancy probabilities were observed to be consistently high and there was no marked change in occupancy state between the fluctuating flow regime and experimental steady flow regimes. This is interesting because it had been hypothesized that stabilized steady flow would elicit a population level response causing occupancy patterns to change between flow regimes. Because estimates of occupancy probabilities were high, robust relationships between habitat covariates and humpback occurrence were not able to be estimated. Detection probabilities exhibited considerable heterogeneity reflecting differences among survey, size class and habitat covariates.

The magnitude of discharge releases from Glen Canyon Dam between the fluctuating flow regime and steady flow regimes may not have been sufficient to elicit a response in humpback chub populations. The implementation of Modified Low Fluctuating Flow (MLFF) policy from Glen Canyon Dam in 1996 reduced the diel range in discharge and reduced the frequency of low flow events (Gloss et al. 2006) effectively minimizing large daily contrasts in
discharge from Glen Canyon Dam. Despite differences in discharge during field surveys from 2009 to 2011 the ranges of flows observed in this study were within the operational constraints of MLFF. If the contrasts in flow regimes had been greater humpback chub responses to steady flow experiment may have been observed.

The spatial location of the study area may have contributed to the lack of a response to flow variation by humpback chub. Korman et al. (2004) modeled humpback chub habitat use over a range of discharges and found that areas which were comprised of steep angle shoreline such as talus and debris fan habitat types were more robust to changes in discharge than low-angle homogenous shorelines such as sandbars. About 60% of our study area was comprised of talus and debris fan shorelines and geomorphically defined by eddy-fan complexes. The high structural complexity of shorelines in my study area may act as a refuge from discharge fluctuations which allowed humpback chub to be resistant to changing river stage (Korman et al. 2004). We postulate that the general habitat attributes available to juvenile humpback chub likely did not change enough between the fluctuating flow and the steady flow regimes to necessitate a shift in habitat use. While there is no natural analog in river systems to a fluctuating flow regime, minor peaking operations such as modified low fluctuating flow operations from Glen Canyon Dam (but see work related to fish population responses to hydropoeaking in other systems i.e., Moog 1993; Freeman et al. 2001; Scruton et al. 2005; Murchie et al. 2008) may not influence how chub use habitat significantly. However, this could vary spatially and temporally.

The timing of the steady flow experiment may have also influenced the lack of response by humpback chub to the steady flow regimes. Different experimental designs that alternated the timing of the steady flows could be considered in the future to assess how flow regime and season interact to influence occupancy. This may be important in some seasons when occupancy may be influenced by changes in density related to emigration of juvenile humpback chub from the Little Colorado River. The timing of the experimental flows we assessed may also explain why Ross and Grams (2011) found negligible differences in nearshore temperatures between the fluctuating and steady flow during the 2010 field season. A previous steady flow experiment implemented in the summer of 2000 demonstrated that nearshore water
temperatures do warm considerably if the discharge releases are low and the timing allows for maximum absorption of solar radiation (i.e. summer; Trammel et al. 2002). To better understand the interaction between Glen Canyon Dam operations and humpback chub populations, future flow experiments should give careful consideration to the magnitude of discharge released from Glen Canyon Dam, the spatial location where fish monitoring will occur and the timing of discharge releases.

In this study no strong relationships between habitat covariates and site occupancy were established. This suggests that of the parameters measured and modeled no single variable was a strong predictor of whether a habitat will be occupied by humpback chub. Past research by Converse (et al. 1998) suggested that juvenile humpback chub selected vegetated, talus and debris fan habitat attributes while generally avoiding cobble, bedrock and sandy habitats attributes. Differences in observed habitat relationships between this study and prior research may be a result of multiple factors. First, abundance of humpback chub has increased in recent years (Coggins et al. 2011; Vanhaverbeke et al. 2013) from the time period of the Converse et al. (1998) study potentially influencing habitat use patterns. In companion papers (Dodrill et al. in-review, Finch et al. in-review) we document recent trends in humpback chub abundance and document increasing trends in abundance in each year from 2009-2011. It is possible that the lack of habitat and occupancy relationships could be because most available nearshore habitats within our study area were being used across the densities of fish in different habitats (Dodrill et al. in-review). Factors governing humpback chub occupancy may occur at larger spatial scales than was considered in this study. This is important because the absence of relationship between different habitat types and humpback chub occupancy may not persist in areas with different humpback chub densities or habitat types that are more sensitive to changes in river stage than our study reach. In the future, the use of occupancy models as an analytical technique may be most useful when combined with a generalized linear modeling approach that estimates habitat covariate influence on humpback chub abundance. This nested approach would allow occupancy models to determine the scale at which chub occurrence varies and the generalized linear model would identify factors that are related to fish density.
Second, previous assessments of habitat use of humpback chub in Grand Canyon did not incorporate an estimation of detectability; therefore it is uncertain if the observed patterns of catch are in fact differences in habitat use or whether sampling was more effective in particular habitats (Valdez and Ryel 1995, Converse et al. 1998, Hubert and Fabrizio 2007). Detection probability in this study varied among humpback chub size category and survey suggesting size selectivity of the gear and temporal variation in humpback chub available for capture. Habitat characteristics including mean site depth and substrate heterogeneity were also observed to influence detection probability depending on the gear type used for sampling. Understanding how detection changes temporally, with fish size and site habitat characteristics will be useful for future and research and monitoring activities.

Third, humpback chub likely undergo ontogenetic habitat shifts from using nearshore habitats as juveniles to large recirculating eddies as adults (Gorman and Stone 1999, Gerig et al. in-review). In our study we found hoopnet detectability was increased at intermediate depths 5-10 m from shore and most effectively sampled humpback chub size classes between 40-80 mm and 81-150 mm. Larger humpback chub having undergone their ontogenesis may move in and out of the effective sampling area of an individual hoopnet while occupying an area larger than an individual habitat site. We found that juvenile chub between 40-80 mm, which reside primarily in nearshore habitats, exhibited higher detection probabilities when sampled using boat electrofishing (which is designed to sample nearshore areas) and in habitat sub-units with increased substrate heterogeneity. Sites with higher substrate heterogeneity likely have increased interstitial space between rocks and boulders and may serve as refuge habitats from predators, offer low velocity areas to minimize energy costs, or relatively constant habitat area across a range of river stage levels.

We observed temporal variation in detection probabilities of humpback chub between sampling trips within and between years. Considering sampling survey as a covariate likely integrates important biotic and physical factors that differed between trips such as season, turbidity, and humpback chub abundance that can act in concert to influence detection probability. As an example, the large reduction in detection probability (and catch) in October of 2009 may be related to turbidity conditions as turbidity during the October survey of 2009
was the lowest observed during the course of the study (mean=4 NTU). In response to low
turbidity conditions, humpback chub may reduce movement and seek physical cover in talus or
debris fan shorelines where habitat complexity is high, move to deeper water which may
position the fish outside of the effective sampling area for our two gear types, or potentially
eminate from our sampling site due to a perceived increase in predation risk caused by clear
water (Stone 2010). Stone (2010) suggested that humpback chub in the Little Colorado River
may use hoopnets as a type of habitat cover during periods of low turbidity. We did not see
similar increases in hoopnet catch rate, detection probability or occupancy in relation to
turbidity.

We did observe increases in detection probabilities from July to September in both 2010
and 2011, and these rates plateaued or decreased from September to October. Detectability is
influenced by increases or decreases in abundance (MacKenzie and Royale 2005) and we did
document increasing abundance through time for juvenile humpack chub in each year 2009-
2011 (Finch et al. in-review; Dodrill et al. in-review). Flooding from the Little Colorado River
may influence emigration of juvenile humpack chub to the mainstem Colorado River. During
our study we observed more frequent flood events in the Little Colorado River during 2010 and
smaller events in 2011 which may have influenced detectability in this study compared to the
primarily baseflow levels of the Little Colorado River during our sampling in 2009.

Conclusions

We found that humpback chub occupancy of different habitat types was consistently
high within the NSE study reach in the mainstem Colorado River during 2009-2011 and there
were no apparent changes in occupancy between the fluctuating and the steady flow
treatments. Similarly, analysis of humpback chub habitat selection and movement in this reach
suggests that the steady flow regimes did not significantly influence their spatial distribution
and movement rates (Gerig et al. in-review). The lack of significant response to the flow
experiment is important. Previous researchers have noted that it is difficult to derive
predictable relationships between hydrologic change and fish response and that fish responses
to management actions are often counterintuitive (Pine et al. 2009, Poff and Zimmerman 2010,
Branford et al. 2011). The adoption of the natural flow paradigm (Poff et al. 1997) which
suggests that as flow regime is incrementally restored incremental changes in ecosystem function will be observed, is an intuitively appealing paradigm to guide management in regulated river systems. However, our results suggest small changes in flow regime such as a stabilization of flow do not significantly alter how humpback chub use and select habitat.
Table 1. Covariates thought to influence probability of occupancy and probability of detection of fish in Colorado River in Grand Canyon.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Inference to Occupancy (Ψ)</th>
<th>Inference to Detection (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>Ψ varies with mean depth of site</td>
<td>p varies with mean depth of site</td>
</tr>
<tr>
<td>Substrate small</td>
<td>Ψ varies with proportion small subs. in site</td>
<td>p varies with proportion small subs. in site</td>
</tr>
<tr>
<td>Substrate medium</td>
<td>Ψ varies with proportion medium subs. in site</td>
<td>P varies with proportion medium subs. in site</td>
</tr>
<tr>
<td>Substrate large</td>
<td>Ψ varies with proportion large subs. in site</td>
<td>p varies with proportion large subs. in site</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>Does not influence Ψ</td>
<td>p is influenced by turbidity level</td>
</tr>
<tr>
<td>Habitat type</td>
<td>Ψ varies by habitat type of site</td>
<td>p varies with habitat type of site</td>
</tr>
<tr>
<td>Hydraulic type</td>
<td>Ψ varies by hydraulic type</td>
<td>p varies with hydraulic type</td>
</tr>
<tr>
<td>Reach (EF)</td>
<td>Ψ varies spatially by reach for electrofishing</td>
<td>p varies spatially by reach for EF</td>
</tr>
<tr>
<td>Trip</td>
<td>Ψ varies temporally between trips</td>
<td>p varies temporally between trips</td>
</tr>
<tr>
<td>Year</td>
<td>Ψ varies with temporal variation in abundance</td>
<td>p varies with temporal variation in abundance</td>
</tr>
<tr>
<td>Flow Regime</td>
<td>Ψ varies with dam operations</td>
<td>p varies with dam operations</td>
</tr>
</tbody>
</table>
Table 2. Species percent composition and total catch during 2009 – 2011 from repeated hoopnet surveys in the Colorado River in Grand Canyon.

<table>
<thead>
<tr>
<th>Species</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBH</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>BHS</td>
<td>0.04</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>BNT</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>CCF</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>CRP</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>FHM</td>
<td>0.05</td>
<td>0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>FMS</td>
<td>0.03</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>HBC</td>
<td>0.84</td>
<td>0.81</td>
<td>0.80</td>
</tr>
<tr>
<td>PKF</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>RBT</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>RSH</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>SPD</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Total Catch</td>
<td>512</td>
<td>623</td>
<td>841</td>
</tr>
</tbody>
</table>

Table 3. Species percent composition and total catch during 2009 – 2011 from repeated electrofishing surveys in the Colorado River in Grand Canyon.

<table>
<thead>
<tr>
<th>Species</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBH</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>BHS</td>
<td>0.04</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>BK2</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>RNT</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>CCF</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>CRP</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>FHM</td>
<td>0.52</td>
<td>0.42</td>
<td>0.68</td>
</tr>
<tr>
<td>FMS</td>
<td>0.06</td>
<td>0.11</td>
<td>0.04</td>
</tr>
<tr>
<td>GSF</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>HBC</td>
<td>0.23</td>
<td>0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>PKF</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>RBT</td>
<td>0.05</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>RSH</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>SPD</td>
<td>0.08</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>STB</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total Catch</td>
<td>1336</td>
<td>1604</td>
<td>2155</td>
</tr>
</tbody>
</table>
Table 4. Ranking of site occupancy models used to determine covariates influential in the
detection of humpback chub captured in repeated hoopnet surveys from 2009-2011.

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>AIC wt</th>
<th>K^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ψ(.), p(Size+Trip+Depth+Depth²)</td>
<td>20733</td>
<td>0</td>
<td>0.9998</td>
<td>18</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Trip+Depth)</td>
<td>20751</td>
<td>17.13</td>
<td>0.0002</td>
<td>17</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Trip+Large)</td>
<td>20755</td>
<td>21.58</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Trip+Medium)</td>
<td>20772</td>
<td>38.66</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Trip+Habitat)</td>
<td>20773</td>
<td>39.84</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Trip+Small)</td>
<td>20775</td>
<td>41.17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Trip+Hydraulic Type)</td>
<td>20778</td>
<td>44.4</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Trip)</td>
<td>20783</td>
<td>49.37</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Trip+NTU)</td>
<td>20783</td>
<td>49.42</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Year+Flow Regime)</td>
<td>20988</td>
<td>254.09</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Flow Regime)</td>
<td>21058</td>
<td>324.71</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Year)</td>
<td>21087</td>
<td>353.63</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Ψ(.), p(Size)</td>
<td>21126</td>
<td>392.19</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Ψ(.), p(.)</td>
<td>21202</td>
<td>468.54</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

^a = # of parameters
Table 5. Ranking of site occupancy models used to determine covariates influential in the detection of humpback chub captured in repeated electrofishing surveys from 2009-2011.

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>AIC wt</th>
<th>K^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ψ(.), p(Size+Reach+Trip+Medium)</td>
<td>9216.44</td>
<td>0</td>
<td>0.7837</td>
<td>18</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Trip+Small)</td>
<td>9219.19</td>
<td>2.75</td>
<td>0.1981</td>
<td>18</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Trip+Depth)</td>
<td>9225.49</td>
<td>9.05</td>
<td>0.0085</td>
<td>18</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Trip +Habitat)</td>
<td>9226.01</td>
<td>9.57</td>
<td>0.0065</td>
<td>20</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Trip+Depth2)</td>
<td>9227.47</td>
<td>11.03</td>
<td>0.0032</td>
<td>19</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Trip+Hydraulic Type)</td>
<td>9237.83</td>
<td>21.39</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Trip+Large)</td>
<td>9247.31</td>
<td>30.87</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Trip+NTU)</td>
<td>9248.94</td>
<td>32.5</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Trip)</td>
<td>9250.49</td>
<td>34.05</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Season)</td>
<td>9329.09</td>
<td>112.65</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach+Year+Flow Regime)</td>
<td>9339.02</td>
<td>122.58</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Reach)</td>
<td>9353.4</td>
<td>136.96</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Year)</td>
<td>9431.67</td>
<td>215.23</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Ψ(.), p(Size+Flow Regime)</td>
<td>9433.62</td>
<td>217.18</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Ψ(.), p(Size)</td>
<td>9434.05</td>
<td>217.61</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Ψ(.), p(.)</td>
<td>9590.02</td>
<td>373.58</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

^a = # of parameters
Table 6. Ranking of site occupancy models from repeated hoopnet surveys from 2009-2011 used to determine covariates influential in to the occupancy probability of humpback chub in Grand Canyon.

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>AIC wt</th>
<th>K&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Psi(\text{Trip+Size}), \ p(\text{Best})$</td>
<td>20703.45</td>
<td>0.00</td>
<td>0.5729</td>
<td>31</td>
</tr>
<tr>
<td>$\Psi(\text{Trip+Size+Hydraulic Type}), \ p(\text{Best})$</td>
<td>20704.23</td>
<td>0.78</td>
<td>0.3879</td>
<td>32</td>
</tr>
<tr>
<td>$\Psi(\text{Trip}), \ p(\text{Best})$</td>
<td>20708.88</td>
<td>5.43</td>
<td>0.0379</td>
<td>29</td>
</tr>
<tr>
<td>$\Psi(\text{Trip+Size+Small}), \ p(\text{Best})$</td>
<td>20716.76</td>
<td>13.31</td>
<td>0.0013</td>
<td>26</td>
</tr>
<tr>
<td>$\Psi(\text{Trip+Size+Medium}), \ p(\text{Best})$</td>
<td>20718.03</td>
<td>14.58</td>
<td>0.0007</td>
<td>26</td>
</tr>
<tr>
<td>$\Psi(\text{Year+Flow Regime+Size}), \ p(\text{Best})$</td>
<td>20719.10</td>
<td>15.65</td>
<td>0.0002</td>
<td>26</td>
</tr>
<tr>
<td>$\Psi(\text{Trip+Size+Large}), \gamma(.), \ p(\text{Best})$</td>
<td>20720.99</td>
<td>17.54</td>
<td>0.0002</td>
<td>26</td>
</tr>
<tr>
<td>$\Psi(\text{Trip+Size+Depth}), \ p(\text{Best})$</td>
<td>20721.09</td>
<td>17.64</td>
<td>0.0001</td>
<td>26</td>
</tr>
<tr>
<td>$\Psi(\text{Year+Flow Regime}), \ p(\text{Best})$</td>
<td>20723.71</td>
<td>20.26</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>$\Psi(\text{Flow Regime+Size}), \ p(\text{Best})$</td>
<td>20724.12</td>
<td>20.67</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>$\Psi(\text{Flow Regime}), \ p(\text{Best})$</td>
<td>20728.38</td>
<td>24.93</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>$\Psi(\text{Year+Size}), \ p(\text{Best})$</td>
<td>20732.05</td>
<td>28.60</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>$\Psi(.), \ p(\text{Best})$</td>
<td>20733.44</td>
<td>29.99</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>$\Psi(\text{Year}), \ p(\text{Best})$</td>
<td>20736.16</td>
<td>32.71</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>$\Psi(\text{Trip+Size+Habitat}), \ p(\text{Best})^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Psi(\text{Trip+Size+Depth+Depth}^2), \ p(\text{Best})^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> = # of parameters, * model did not converge
Table 7. Ranking of site occupancy models from repeated electrofishing surveys from 2009-2011 used to determine covariates influential to the site occupancy of humpback chub in Grand Canyon.

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>AIC wt</th>
<th>K&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ψ(Year+Flow Regime+Size), p(Best)</td>
<td>9160.19</td>
<td>0</td>
<td>0.2189</td>
<td>24</td>
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<sup>a</sup> = # of parameters
Figure 1. Map of the Colorado River in Grand Canyon, AZ. Our study site was between Heart Island (RKM 125) to Lava Chuar Rapid (RKM 131.2) indicated in the white box within the map.
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Gunn, J. M., and R. Sein. Effects of forestry roads on reproductive habitat and exploitation of lake trout (*Salvelinus namaycush*) in three experimental lakes. Canadian Journal of Fisheries and Aquatic Sciences, 57(S2), 97-104.


*in-press documents are provided in this report as separate chapters*
Nearshore Habitat Use Patterns of Juvenile and Small-Bodied Native Fish in the Colorado River, Grand Canyon.

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Abstract

Many management actions in aquatic ecosystems are directed at restoring or improving specific habitats in order to benefit populations of fish or other aquatic species. In the Grand Canyon reach of the Colorado River, experimental flow operations as part of the Glen Canyon Dam Adaptive Management Program often consider the creation and maintenance of sandbars and associated backwater habitats as critical for juvenile native fish conservation. These habitats are thought to be warmer and provide areas of improved growth and survival for juvenile native fish. Previous research has documented the occurrence of native fish in backwater habitats, including the federally endangered humpback chub *Gila cypha*, yet little is known about the distribution of fish among other shoreline habitats. This study quantified abundance and density of juvenile humpback chub, bluehead sucker *Catostomus discobolus*, flannelmouth sucker *Catostomus latipinnis*, and speckled dace *Rhinichthys osculus* in different shoreline habitats (cliff, talus, debris fan, sand, and backwater). Backwaters contained the highest densities of native fish compared to other habitats, however they supported a small proportion of total abundance in the study area because the spatial extent of this habitat was very limited. Abundance of juvenile humpback chub was consistently highest in talus habitats and lowest in backwater habitats. Thus management actions directed at manipulating backwaters will likely only affect a small proportion of the overall abundance of native fish in this reach of the Colorado River.

Key Words: Colorado River, Hierarchical Bayesian Models, Gila cypha, Shoreline Habitat Use, Controlled Floods, River Restoration
Introduction

Globally, riverine ecosystems are highly altered with some of the most common modifications resulting from river regulation following the construction of dams (Dynesius and Nilsson 1994; Nilsson et al. 2005). Increasingly, conservation actions are directed to restore riverine ecosystems by mimicking elements of historical flow patterns to reconnect or create riparian and aquatic habitats (Stromberg 2001; Bednarek and Hart 2005; Bernhardt and Palmer 2011). Proponents argue that restoring habitat diversity in river ecosystems will result in increased biodiversity (Ward and Tockner 2001; Tew et al. 2004; Palmer et al. 2010) and facilitate the recovery of threatened or endangered species (Katz et al. 2007). Restoration of habitats identified as critical for species persistence is a central tenet of recovery efforts for endangered species in both the US and Canada (U.S. Endangered Species Act; Canadian Species at Risk Act).

Since the 1930s, the Colorado River has been extensively modified to develop water projects to meet water and hydroelectric needs of the southwestern US, creating one of the most regulated and highly controlled rivers on Earth (Fradkin 1984). The completion of Glen Canyon Dam (GCD) in 1963 resulted in a suite of physical changes to the downstream riverine environment that greatly influenced the ecology of the Colorado River ecosystem in Grand Canyon (Topping et al 2000, Gloss and Coggins 2005, Kennedy and Gloss 2005). Following completion of GCD, temperature and sediment regimes of the Colorado River were altered creating cold water temperatures and increased water clarity (Topping et al. 2000; Voichick and Wright 2007). From a fish habitat perspective, these changes in temperature and sediment have been
dramatic. As an example, water temperatures in a pre-dam environment likely ranged from about 0-25°C but post-dam only range from 8-15°C due to hypolimnetic water releases. Historically, about 60 million tons of sediment was transported through Marble and Grand Canyons annually (Topping et al. 2000) however, currently more than 90% of this sediment is retained within Lake Powell upstream of GCD (Topping et al. 2000). One consequence of sediment loss in the Colorado River below GCD has been a decrease in the extent and complexity of shoreline sand deposits which provide habitat for juvenile native fish (Schmidt and Graf 1990; Converse et al. 1998).

Concurrent with these changes in the physical environment in this reach of the Colorado River were large changes in native fish community. For example, while bluehead sucker *Catostomus discobolus*, flannelmouth sucker *Catostomus latipinnis*, and speckled dace *Rhinichthys osculus* are still common in Grand Canyon (Walters et al. 2012), three other native species are extripated or are extremely rare (razorback sucker *Xyrauchen texanus*, Colorado pikeminnow *Ptychocheilus lucius*, bonytail chub *Gila elegans*) and there have been large declines in humpback chub *Gila cypha* populations (Valdez and Ryel 1995; Minckley et al. 2003; Coggins et al. 2006). The decline in humpback chub populations has triggered a significant number of modeling and field projects designed to assess potential reasons for population declines as well as test ideas for aiding in population recovery. While the reasons for the humpback chub population decline, and more recent recovery are uncertain (Coggins et al. 2006; Coggins and Walters 2009), leading hypotheses include (1) physical changes such as loss of required habitats following GCD completion and changes in riverine conditions such as temperature and habitat (Converse et al. 1998; Clarkson and Childs 2000;
Stone and Gorman 2006); and (2) biological changes such as competition with or 
predation by non-native fish (Coggins et al. 2011; Yard et al. 2011).

In 1995, the Environmental Impact Statement (EIS) for the operation of GCD 
proposed ‘beach/habitat building flows’ as a management experiment to rebuild 
sandbars and create aquatic habitat in Grand Canyon (US Department of the Interior, 
1995). Sandbars are highly valued by users and management groups in Grand 
Canyon, not only for the aquatic and riparian habitat that they provide, but as campsites 
for backcountry users as well (Webb 1996). Since 1995, four high-flow experiments 
designed to mimic elements of pre-dam floods have occurred to inform restoration 
activities, including creation and maintenance of sandbars, as part of the Glen Canyon 
Dam Adaptive Management Program (GCDAMP; Patten et al. 2001; Melis et al. 2011).

Controlled floods are currently the main strategy to rebuild sandbars and associated 
backwater habitats in order to reverse declines in these resources to benefit human 
users and potentially biological resources within Grand Canyon, including native fish 
populations (Melis et al. 2011). Beach/habitat building flows are thought to benefit 
native fish including humpback chub by redistributing sand to nearshore areas creating 
backwater habitats that are used by juvenile fish (US. DOI 2008 a, b). Understanding 
operational strategies for sediment conservation and the response of native and non-
native fish populations to controlled floods remains a priority for managers which will 
likely necessitate continued experimental floods (Melis et al. 2012).

In Grand Canyon, backwaters are a habitat type generally created by eddy return 
channels and often occur as isolated areas of low flow associated with sandbars (Rubin 
et al. 1990; Schmidt and Graf 1990). These habitat types are thought to be important
for native fish populations because in the post-dam environment, backwaters generally have low rates of water exchange, and much shallower depths, resulting in seasonally warmer temperatures compared with the main river channel (AZGFD 1996; Behn et al. 2010). Because backwaters may offer warmer water temperatures, and water temperatures are linked to fish growth (Coggins and Pine 2010) and thus fish survival (Lorenzen 1996), backwaters are seen as a potentially important refuge for the conservation of native fish in the highly altered post GCD mainstem Colorado River (Hoffnagle 1996; Trammell et al. 2002).

Long-term fish monitoring has documented the presence of juvenile humpback chub and other small-bodied native and non-native fish species in backwater habitats throughout Grand Canyon (AZGFD 1996; Hoffnagle 2000; Grams et al. 2010). Despite years of research on the physical processes that structure backwater development and persistence, including several experimental floods from GCD, fundamental questions remain related to the role backwaters play in native fish ecology. Determining the distribution of juvenile and small-bodied native fish between backwaters and other available shoreline types remains a key area of management interest. This is due to the potential for management actions such as controlled floods coupled with modification in flow operations from GCD to possibly create and maintain backwater habitats, potentially aiding in the recovery of humpback chub and other species. The objective of this study was to compare abundance and density of juvenile and small-bodied native fish between habitats, with emphasis on backwaters.

In order to quantify fish densities between habitats, we developed a novel multi-species hierarchical Bayesian model. This approach was designed to improve closed
population estimates that may fail or produce unbounded results when dealing with sparse mark-recapture data (Pine et al. 2003). A key attribute is that our modeling strategy allowed for information from more common species with larger numbers of marks and recaptures to inform the estimates of density for less common species. We assumed that captures of small-bodied fish species came from a common distribution, allowing information to be shared between species, and resulting in improved estimates for species with little information. Hierarchical Bayesian models may appeal to managers or researchers interested in improving abundance estimates for rare species, which is often necessary for accurate assessment of threatened or endangered species status.

Methods

Study Site

This study was conducted in the Colorado River downstream of the confluence of the Little Colorado River (LCR) and the Colorado River between 127 river kilometer (rkm) to 132 rkm as measured downstream from Glen Canyon Dam (Figure 1). The largest aggregation of humpback chub is found in this section of the Colorado River (Gorman and Stone 1999) and juvenile humpback chub disperse from the LCR into this area (Robinson et al. 1998). Additionally this reach has a high density of debris fan complexes that potentially form backwaters (Schmidt and Graf 1990) as well as other common habitat types in which to compare habitat use. Sampling occurred four times a year (July, August, September, and October) in three consecutive years (2009, 2010, and 2011).
Fish Sampling in Backwater Habitats

During each sampling trip backwaters were sampled for small-bodied fish with seines (9-25-m in length x 1.2-m height x 3-mm mesh) and block-nets to complete multi-pass depletions (Seber 1982). Sampling was conducted after 2100 h due to fluctuating dam operations which produced changes in stage during July and August. These fluctuations often overtop the sandbars isolating backwaters from the main channel, making these habitats unavailable during many of the day light hours. Prior to seining, backwaters were blocked off from the main channel using a block-net, then a minimum of five seine passes removed fish until the backwater was depleted. After each pass, fish were removed from the net and held, then identified to species, measured, and given identifying visual implant elastomer (VIE) marks or passive integrated transponder (PIT) tags (following Grand Canyon fish sampling protocols, Persons et al. 2013).

Fish Sampling in Other Habitat Types

Boat mounted electrofishing was conducted after dark (1900 to 0300 h) in three sampling sites, each approximately 1.2 km, within the study area to sample the near-shore fish community. Within each site, shorelines were subdivided into individual spatially referenced 50-m segments (hereafter “Habitat Sampling Units” or HSUs) and classified as one of four discrete habitat types (sand, debris fan, talus, cliff, Table. 1) based on existing Geographic Information Systems (GIS) imagery (see below). Each site was repeatedly sampled three times over five day periods (48-h between passes, 2009) or over successive nights (24-h between passes, 2010 and 2011). Two aluminum-hulled boats (4.9-m length) each equipped with a 5000-W generator and a variable-voltage pulsator (Coffelt Mark XXII) were used to slowly (5-10 seconds per
meter of shoreline) sample nearshore areas for small-bodied fish. Fish were processed similarly to fish captured by seining with the exception that fish 40-99-mm TL received a VIE mark corresponding to sampling trip, gear and site. Fish were returned to the HSU from which they were captured.

Available Nearshore Habitat

To account for possible changes in the availability of nearshore aquatic habitats, each individual HSU was classified as a discrete habitat type during each trip during 2009 and 2010. This was accomplished using habitat maps derived from GIS which were verified in field. The linear shoreline length of each habitat type (excluding backwater) was determined by summation of the individual HSU lengths. The shoreline lengths of backwaters were estimated during each sampling occasion and summed across the entire study area. Generally shoreline habitats consisted of multiple HSUs of the same habitat type forming continuous stretches. During 2009 and 2010 sampling, very little change occurred in the overall proportion of each habitat type within the study site so with the exception of backwater habitats, we assumed habitat proportions in 2011 were similar to 2009-2010. Due to higher flow volumes that occurred in July, August, September and October 2011, backwater habitat was unavailable in the sampling site.

Hierarchical Bayesian Models

We used hierarchical Bayesian models (HBM) to estimate fish density and abundance within the study area using both removal and mark-recapture methods. Removal models were used for the samples collected in backwaters and mark-recapture models were used for electrofishing samples. Removal and mark-recapture
methods have long been used in animal ecology (i.e., Leslie and Davis 1939) to adjust
animal counts for individuals that were not detected and thus provide unbiased
estimates of animal abundance with respect to detection (Williams et al. 2002).
Recently, these methods have been implemented in a hierarchical Bayesian framework
(Rivot et al. 2008; Korman et al. 2010; Bohrmann et al. 2012) which allows greater
modeling flexibility and accurate assessment of model uncertainty (Royle and Dorazio
2006; Cressie et al. 2009).

The hierarchical modeling approach used assumes that sampling units (mark-
recapture or removal sites) are exchangeable (similar but not identical), and parameters
that describe processes in each sampling unit are generated from the same stochastic
processes. Parameter values for each unit are expected to be similar and estimated
from a common distribution, as opposed to assuming there is no variation in parameters
across sites (a ‘pooled model’) or that parameters are independent (Royle and Dorazio
2008; Korman et al. 2010). The parameters that define this common distribution are
referred to as hyperparameters and are estimated along with the site specific
parameters. Hierarchical models provide a compromise, often referred to as partial-
pooling, which is often advantageous when within group data is sparse. For example,
site specific parameters can be grouped together resulting in ‘borrowing strength’
between effects that pulls extreme or low precision individual estimates towards the
group mean, often resulting in greater precision (Kery and Schaub 2012). Additionally,
hierarchical models explicitly consider model processes at multiple levels and allow
proper accounting of complex variance structures, often a hallmark of biological
systems (Uriarte and Yackulic 2009).
The mark-recapture model used to make inferences for shorelines sampled with electrofishing assumes that fish abundance ($N$) follows a Poisson distribution, such that,

$$N_{s,h,t,r} \sim \text{Pois}(\lambda_{s,h,t})$$

where $s$ denotes the index for species, $h$ denotes the index for habitat, $t$ denotes the index for sampling trip, and $r$ denotes the index for sampling reach (See Table 2). The rate parameter, $\lambda_{s,h,t}$, for the Poisson distribution is assumed to have a log-linear relationship with covariates,

$$\log(\lambda_{s,h,t}) = \log(A_{h,t,r}) + \alpha_{s,h} + \beta_{s,t} + \varepsilon_s$$

where $\alpha$ is a fixed effect indexed for each species ($s$) and habitat ($h$), $\beta$ was included as a random effect that specifies a random trip deviate drawn from a normal distribution for each species (i.e., $\beta_{s,t} \sim \text{Normal}(0, \tau_s)$), and the deviate $\varepsilon$ is drawn from a normal distribution centered on zero with species specific precision ($\tau_s$). We included $\varepsilon$ to account for variation within each species beyond that which is expected from the Poisson distribution (i.e., to account for overdispersion). Density is scaled to abundance by including the log of area sampled ($A$) as an offset. The area sampled varies between each habitat ($h$), trip ($t$), and reach ($r$).

Detection ($\theta$) was modeled on a logit-link scale,

$$\text{Logit}(\theta_{s,h,t}) = \gamma_{s,h} + \delta_{s,t}$$

with a random effect ($\gamma$) for each species ($s$) and habitat ($h$) centered on mu ($\mu$), and a random trip effect ($\delta$) for each species centered on zero. Catch ($C$) of unmarked fish and recaptures ($R$) of marked fish across passes but within a sampling reach ($r$) were treated as Poisson random variables,

$$C_{s,h,t,r,p} \sim \text{Pois}(N_{s,h,t,r}, \theta_{s,h,t})$$
with the product of abundance (N) and detection (θ) for each species (s), habitat (h),
and trip (t) strata defining the expected catch. The expected recaptures was the
product of marks available (M) on a given pass (p) and detection (θ) for each strata.
Abundance each trip for each species was estimated by taking the habitat and species
specific density estimates (scaled to fish per one meter of shoreline) and adding any trip
and species specific deviates, then multiplying by the amount of available habitat
measured each trip.

The removal HBM used to gain inference from the seine sampling in backwaters
follows the methods outlined in Royle and Dorazio 2006, where abundance follows a
Poisson distribution. Similar to the mark-recapture model, the log of backwater area (A)
for each trip was included as an offset, and we modeled fish abundance (N),

\[ N_{s,t} \sim \text{Pois}(\lambda_{s,t}) \]

\[ \log(\lambda_{s,t}) = \log(A_t) + \eta_s + \kappa_{s,t} \]

where s denotes the index for species, and t denotes the index for trip. Covariates are
modeled using a log or logit-link function for density or detection probability, respectively
(See Table 2). Catch (C) for each pass (p) of the removal seining was considered a
binomial random variable,

\[ C_{s,t,i,p} \sim \text{binom}(\theta_{s,t}, Z_{s,t,p}), \text{ where} \]

\[ Z_{s,t,p} = N_{s,t}, \text{ for p}=1, \text{ and} \]

\[ Z_{s,t,p} = N_{s,t} - \sum_{x=1}^{p-1} C_{s,t,i,x} \text{ for p}>1 \]

with detection probability (θ) and fish abundance indexed by species (s) and trip (t). A
fixed species effect and a random trip deviate were included for detection probability
The removal model assumes that detection is constant between passes and individual fish. Estimates of abundance across the entire study area during each trip were made by taking the species specific density estimates and adding any trip specific deviates, and then expanded by the amount of available backwater habitat.

Data from individual HSUs were too spare for precise mark-recapture estimates, therefore captures, recaptures, and marks were pooled by shoreline habitat type and sampling site (see above) prior to modeling. The mark-recapture HBM used is a closed population model with standard assumptions for this model type (Pine et al. 2003). The assumption of site closer between shoreline types is reasonable considering the spatial arrangement of HSUs (generally continuous stretches of one type) and the short duration of sampling (3 to 5 days). In addition, the closure assumption was assessed by examining movement patterns of fish in the study site (Pine et al. 2011) and assessments of age-0 fish movement from other rivers, suggests that over short time-scales that this assumption is likely valid (Mitro and Zale 2002; Dunham et al. 2002).

For both the mark-recapture and removal models all priors were weakly informative (i.e., prior for $\alpha$ is uniform (-7, 7)). Deviance Information Criterion (DIC), a model comparison framework similar to Akaike Information Criteria (AIC), was used to compare alternative model structures to examine the effects of shoreline habitats on fish density estimates (Spiegelhalter et al. 2002). Generally differences in DIC between models were small and inferences were similar between model structures with respect to densities in backwaters and other available habitats (Appendix 1, Table A1, Figure A1). We chose to present a model that included the habitat effect for both fish density and detection to illustrate any possible patterns of habitat use across sampling trips.
Posterior probability distributions of model parameters were estimated using a Monte Carlo-Markov chain (MCMC) algorithm in WinBUGS (Spiegelhalter et al. 1999). WinBUGS was called using the package R2WinBUGS (Sturtz et al. 2005) in program R (R Development Core Team 2012). Three independent MCMC chains were initiated with different random values and run for 100,000 iterations with a 20,000 iteration burn-in (discarded) and every 10th sample was kept. To assure that the simulation converged on the posterior distributions, we visually inspected MCMC chains for signs of poor mixing and assessed convergence using the Gelman-Rubin diagnostic with values less than 1.1 indicating convergence (Gelman et al. 2004). Model fit was evaluated using posterior predictive checks, by calculating a Bayesian p-value using a chi-squared discrepancy measure (Kery 2010, Table A1). A Bayesian p-value of 0.5 indicates perfect model fit, while values close to 0 or 1 suggest a lack of fit.

Results

The most common shoreline habitats in the study site were talus (~36%) followed by debris fan (~24%), sand (~20%), and cliff (~18%). Backwater habitat made up a small proportion (~1%) of the total available shoreline in the study area during 2009 and 2010 (Table 3). No backwater habitat was available during 2011 due to large flow volumes from GCD. The proportions of each habitat type were consistent across sampling trips in 2009 and 2010 (Table 3).

Both the removal and mark-recapture HBM provided relatively precise estimates of detection probability for each of the sampling methods and gears (Figure 2). By
including trip deviates (removal models) or the species specific trip deviates (mark-
recapture models) the models were flexible enough to account for changing sampling
conditions (turbidity, flow conditions) or changing abundance (following flooding in the
LCR) between trips. Large differences in detection between species were not observed
from the removal sampling of backwaters and estimates of detection were generally
high (Figure 2, A). Juvenile flannelmouth sucker had the highest detection and the
posterior distributions for all species overlapped. The mean of the hyperdistribution for
electrofishing was 0.05 (0.038-0.063 95% CRI) and generally estimates of detection for
individual species and habitats were precise.

Juvenile humpback chub abundance was significantly lower in backwaters
compared to all other habitat types during most of the 2009 and 2010 sampling trips
(Figure 3). Considering shoreline habitats other than backwaters, abundance of
humpback chub was higher in talus shorelines compared to other habitats and this trend
was largely consistent across sampling trips and years (Figure 3). However, uncertainty
in humpback chub abundance estimates was greater in talus when compared to other
shoreline types. In general, abundance was similar between debris fan, sand and cliff
shorelines and 95% credible intervals overlapped. Across all shoreline habitat types
with in the study site, no strong temporal trends were observed for juvenile humpback
chub total abundance (Figure 4). The highest abundance of juvenile humpback chub
was observed in September 2011.

Juvenile bluehead and flannelmouth sucker abundance varied temporally and
between habitats with the highest abundance generally in sand shorelines (Figure 3).
During September of 2009, the point estimate for bluehead sucker abundance was
higher in backwaters than any other habitat type, but credible intervals overlapped with sand shorelines. During several sampling trips, abundance of both sucker species was comparable in backwaters to other shoreline types. Across all habitats within the study site, juvenile bluehead sucker total abundance was generally higher in August and September compared with July and October (Figure 4). Juvenile flannelmouth suckers did not show any strong temporal trends in total abundance across the study site (Figure 4). Speckled dace abundance was consistently higher in debris fan habitat and lowest in backwaters, and credible intervals generally overlapped between months and habitat types (Figure 3). Total abundance of speckled dace across the entire study site was highest during 2010 and abundance generally declined from July through October (Figure 4).

We found that juvenile humpback chub density was higher in backwaters when compared with other shoreline types (Figure 5). This was also true for bluehead sucker, flannelmouth sucker, and speckled dace, where higher densities were often found in backwaters, although credible intervals for backwater estimates were generally large and overlapped with some habitat types. Bluehead sucker, and flannelmouth sucker exhibited significantly higher densities in backwaters when compared to other shoreline types (Figure 5). Across all species, fish density in backwaters varied considerably, with individual trip estimates ranging from 2 to 247 fish/100 m of shoreline. Variation in backwater fish densities was driven in part by uncertainty in detection probability (Figure 2), but also by large variation in fish abundance between sampling trips (Figure 3).

When considering the majority of available habitat, which occurred outside of backwaters, densities of juvenile humpback chub were largely similar between habitat
types and 95% credible intervals overlapped (Figure 5). No large differences in density were observed for speckled dace, or juvenile bluehead and flannelmouth suckers between habitats when excluding backwaters (Figure 5). For many species, density estimates for shoreline types sampled with electrofishing were less variable when compared to density estimates in backwaters. We also observed higher densities of bluehead and flannelmouth sucker in sand habitats, although 95% credible intervals overlapped (Figure 5). Speckled dace densities were higher in debris fan habitats compared with sand, cliff and talus shorelines (Figure 5).

In general, the multi-species mark-recapture model lead to more precise estimates of density by habitat type (Figure 5). This is especially true for bluehead and flannelmouth suckers which had sparse mark-recapture data. Point estimates for both sucker species were generally higher with large 95% credible intervals (particularly the upper 95% bound) under the individual model compared with the multi-species model. This is generally a reflection of low detection probability estimates for these species owing to the small numbers of initial marks and subsequent recaptures. Density estimates for humpback chub and speckled dace were very similar between the individual and multi-species models due to the comparatively more data for these species.

Discussion

While, our results demonstrate that juvenile native fish including humpback chub often occur in high densities in backwaters, these habitats in this area of river only represent a small proportion of the available habitat and thus only a small portion of the
overall native fish abundance. The limited spatial extent and ephemeral nature of backwaters, suggests that this habitat may not be required by humpback chub for population persistence, but when backwaters are available humpback chub may use these habitats in higher densities than surrounding shoreline types. Many surveys of juvenile fish in Grand Canyon have focused on backwaters because of their perceived importance as rearing habitat for juvenile native fish (AZGFD 1996; Hoffnagel 2000). Support for the importance of backwaters came from observations that water temperatures in backwaters are often slightly warmer than surrounding nearshore areas (AZGFD 1996; Trammell et al. 2002) coupled with the belief that temperature may confer a growth advantage to native fish (Clarkson and Childs 2000; Petersen and Paukert 2005). Moreover, investigations of basal resources in backwaters found that availability of prey items for juvenile fish are comparable between backwaters and other habitats (Brouder et al. 1999; Behn et al. 2010). The March 2008 high-flow experiment from GCD showed that during a short time frame post flooding, increases in backwater area were achieved (Grams et al. 2010). Yet, to date, there has been no measurable response in the humpback chub population dynamics that can be attributed to experimental high-flow experiments (Coggins and Walters 2009; Kennedy and Ralston 2011) that are hypothesized benefit native fish by the creation of backwaters (DOI 2008 a, b). The overall distribution of juvenile native fish observed in this study suggests that while native fish often occur in higher densities within backwater habitats, this habitat is so rare that it affects only a small portion of the overall fish populations. Juvenile humpback chub were most abundant in talus and debris fan habitats, which were common habitat types that supported similar fish densities. Point estimates
of density were also slightly higher in these two habitats as compared to other non-backwater habitats, however, the confidence intervals for all non-backwater habitats overlapped. Previous research suggests that if effort were increased to improve precision that these differences might persist. Converse et al. (1998) and Valdez and Ryel (1995) quantified habitat use for subadult humpback chub (<200 mm TL) and found the highest relative densities of fish in vegetated, talus, and debris fan shorelines. These authors suggested that cover (light and velocity refugia) provided by these habitats was primarily responsible for the higher relative fish densities (Converse et al. 1998).

We observed higher densities of both juvenile bluehead and flannelmouth suckers associated with sand shorelines. These results are similar to studies of juvenile bluehead and flannelmouth sucker habitat use in secondary channels of the San Juan River that showed associations with fine substrates as well as high overlap of habitat use with other native juvenile fish (Gido and Propst 1999). The highest density of speckled dace was associated with debris fan habitats, which aligns with previous studies of speckled dace that show this species affinity for higher velocity riffle habitats (Rinne 1992; Gido and Propst 1999). Our understanding of juvenile bluehead sucker, flannelmouth sucker, and speckled dace habitat use comes mostly from studies of small streams (Rinne 1992; Bower et al. 2008) or smaller tributaries of the Colorado River (Gido and Propst 1999). This study adds to this research for these species by documenting patterns of habitat use in the mainstem of a large regulated river. The availability of shoreline habitats for juvenile fish in Grand Canyon is dependent on the dominant discharge and sediment conditions as well as local
Since the closure of GCD, the complexity and extent of sand deposits has decreased (Schmidt and Rubin 1995; but see Goeking et al. 2003), and there is likely an increase in the proportion of more structurally complex shoreline types. We found higher densities of humpback chub and speckled dace in shoreline types with more heterogeneous substrates. In rivers, heterogeneous habitats are thought to provide a variety of benefits to juvenile fish (Pretty et al. 2003), including low velocity refuges that benefit juvenile fish due to decreased energetic losses from swimming (Crook and Robertson 1999), particularly during increased flows (Freeman et al. 2001; Humphries et al. 2002). For drift feeding fish, hydraulic cover may also provide efficient foraging sites (Fausch 1984; Allouche 2002). Structure may also serve as refuge from predation (Allouche 2002) and generally reduces foraging efficiency of predators (Savino and Stein 1982). Although recent research from the Colorado River suggests that predation risk may be higher in more structurally complex habitats (debris fans) compared with a more structurally simple areas (backwaters), under low to intermediate turbidity conditions (Dodrill et al. in review).

Due to the functions that heterogeneous habitats provide to small fish, habitat enhancements in many rivers aim to increase structural complexity with the addition of engineered rock structures (i.e., dikes, rock revetments, Ridenour et al. 2009). Many reports document increased relative abundance of juvenile fish associated with these structures (Ridenour et al. 2009; White et al. 2010; Hartman and Titus 2010) and advocate habitat enhancements to mitigate anthropogenic changes in large rivers (Hartman and Titus 2010). Responses to structural enhancement may be species dependent, likely strongest for species that generally inhabit lentic (i.e., family...
Centrarchidae) or off-channel portions of lotic systems (Hartman and Titus 2010), and likely not as strong for humpback chub or other native fish, which have an evolutionary history with large, turbulent, canyon bound river reaches. As the proportion of complex shoreline types likely increases due to continued loss of sediment in Grand Canyon, it is uncertain which species may benefit most. Understanding how biological factors (predation risks, food availability) differ between habitats and ultimately affect growth and survival, will help to resolve some uncertainty related to changing shoreline habitats in the Colorado River.

Some care should be taken in inferring that habitats with higher densities are actually better habitats (van Horne 1983). While higher densities often correlate with measures of fitness (Bock and Jones 2004; Perot and Villard 2009), the relationship between density and habitat quality can breakdown when either environmental factors (i.e. temporally dynamic habitat) or species characteristics (i.e. social structure) led to high densities in low quality habitats (van Horn 1983). Frequently, intraspecific and interspecific competition can occur at high densities in fish populations, leading to density dependent growth that can ultimately affect recruitment dynamics (Post et al. 1999; Lorenzen and Enberg 2002). Additionally, habitat use patterns may be density dependent, for example, during times of increased density, dominant individuals may force subdominants into low-quality habitats, so that individuals occupy a wider range of habitat types than at low densities (Rosenfeld et al. 2005). In summary, caution should be exercised in interpreting high densities of native fish found in backwaters as a sign of high quality habitat without additional lines of inference. Continued monitoring of habitat use across various conditions (i.e., fish densities) coupled estimates of quantities more
closely linked to fitness (i.e. growth and/or survival) would greatly benefit the 
management of native fish species in Grand Canyon, by providing further inference in 
defining habitat requirements.

Heterogeneity in capture probability either between habitat types or between 
passes within a habitat can potentially bias estimates and lead to misleading inferences 
concerning differences in density and abundance. Including habitat specific detection 
probabilities allowed us to control bias cause by variation in detection probability 
between habitats. For removal methods, violating the assumption of constant capture 
probability across passes, results in an overestimation of capture probability and an 
underestimation of abundance (Peterson et al. 2004; Rosenberger and Dunham 2005). 
We used a minimum of five passes for the removal sampling, following Riley and 
Fausch (1992) who suggest increasing the number of passes to decrease potential bias 
in removal sampling. The potential for overestimation of capture probability exits in the 
removal model, however due to the large differences often observed between 
backwaters and other habitats, this bias would generally not affect the conclusions of 
this study.

The multi-species approach used in this analysis specified that detection 
probabilities come from a shared distribution, allowing information to be shared between 
fish species. This resulted in more realistic estimates of density in situations where 
other types of closed estimators would generally fail (or produce unbounded estimates). 
This required the assumption that detection between species is exchangeable. We feel 
that this assumption is justified given the small size of fish captured in electrofishing, 
and our observations of small-bodied fish responses to electrofishing in the Colorado
River. In particular, the multi-species models increased the precision of the juvenile bluehead and flannelmouth sucker estimates, often making more biologically reasonable estimates possible. Application of multi-species models that borrow information from more common species may be particularly useful in estimating abundance of rare species, such as threatened or endangered species, which are often the focus of management or conservation efforts (MacKenzie et al. 2005). Often monitoring programs of fish species in large rivers incorporate different sampling gears across different habitats (Schloesser, et al. 2012), and hierarchical formulations of mark-recapture or occupancy models allows for the greater flexibility (i.e. efficient incorporation of random effects, Royle and Link 2002) needed to effectively address inference problems in these systems. Additionally, as the need for assessment of community structure grows, methods that integrate information from different gears and taxa is essential for informing management and conservation actions (Lauretta et al. 2013).

Management Implications

Characterizing the distribution of juvenile native fish among shoreline habitats is highly informative for assessing management experiments directed at manipulating physical conditions to benefit native fish populations within Grand Canyon. In regulated rivers, reestablishing elements of the natural flow regime is a cornerstone of restoration approaches (Stromberg 2001; Arthington et al. 2006). In Grand Canyon, controlled floods are one management approach implemented to restore historic habitat features, such as backwaters, in order to benefit native species (US DOI 1995; US DOI 2008a).
Over short timescales, controlled floods have been shown to increase the extent of backwater habitats through building sandbars (Grams et al. 2010). Yet, sandbars are unstable and can change in response to relatively small changes in discharge, making backwaters largely ephemeral and at least in our study area the amount of backwater habitat available is limited (Hazel et al. 2006; Grams et al. 2010). Our findings suggest that, from a population perspective, abundance of juvenile humpback chub in backwater habitat was generally low, likely as a function of the limited habitat availability (~1% available habitat) within the study area. In comparison, juvenile humpback chub abundance was highest in structurally complex habitats including talus which represents approximately 36% of the available shoreline habitat. This suggests that experimental floods designed to create new or expand existing backwaters will likely only affect a small proportion of the juvenile native fish population in this reach of the Colorado River. Whether these same patterns of habitat use persist in areas outside of the study area, seasonally, or at contrasting densities, is a key area for future work in Grand Canyon. Declines in aquatic resources, such as native fish faunas, within large rivers systems resulting from flow modification are widely recognized (Bunn and Arthington 2002; Aarts et al. 2004; Poff and Zimmerman 2010). Creating aquatic habitats with managed dam releases is a common approach to restore degraded habitats and benefit populations of riverine species (Robinson and Uehlinger 2008; Kieman et al. 2012). To date in Grand Canyon, there has been no measurable effect on native fish populations from the previous experimental floods (Kennedy and Ralston 2011). In fact controlled floods may negatively impact native fish populations due to increased abundance of rainbow trout below GCD that may migrate downstream and prey upon or compete with
native fish (Coggins 2008; Coggins et al. 2011; Yard et al. 2011; Korman 2012).

Successful management may need to consider other approaches, either alone or in concert, such as thermal modifications, predator suppressions, or sediment augmentation in order to restore populations of native fish in Grand Canyon. This research demonstrates the value of detailed monitoring of native fish populations downstream from GCD to inform future management actions. By directly assessing native fish responses to management actions, stronger and more timely inferences can be made to guide management of the Colorado River. Riverine ecosystems are complex and system responses to management can be difficult to predict or appear counterintuitive (Pine et al. 2009), therefore direct monitoring of biological responses induced by management may prove to be an indispensable tool for managers and researchers alike.


Behn, K. E., T. A. Kennedy, and R. O. Hall, Jr. 2010. Basal resources in backwaters of the Colorado River below Glen Canyon Dam--effects of discharge regimes and comparison with mainstem depositional environments.


Bureau of Reclamation, Upper Colorado Regional Office, Salt Lake City, Utah.


Table 1. Definitions of habitat types, modified from Converse et al. (1998).

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Backwater</td>
<td>Areas of low velocity water that are partially isolated from the main channel, often in the lee of an emergent reattachment sandbar.</td>
</tr>
<tr>
<td>Sand</td>
<td>Areas of predominantly exposed sand. Beaches can have very steep banks or be very flat.</td>
</tr>
<tr>
<td>Debris Fan</td>
<td>Debris, predominantly boulder, transported from a tributary during a flooding event. It is characterized by boulders with some degree of embeddedness, intermittent sand beaches, and a small percentage of gravel. The angle of repose is generally flatter than that of talus.</td>
</tr>
<tr>
<td>Talus</td>
<td>Colluvium, predominantly boulder, deposited by rockfall or rockslide activity on the canyon walls. It is characteristically not embedded and has a steeper angle of repose than a debris fan. Debris is more angular as inferred by its process of transportation.</td>
</tr>
<tr>
<td>Cliff</td>
<td>Any rock that is in its original location and has not been transported or broken up by any means. This includes shear walls and laterally or vertically emerging ledges.</td>
</tr>
</tbody>
</table>
Table 2. Definition of variables and indexes in the hierarchical Bayesian model used to estimate density for electrofishing (mark-recapture model) and backwater seine samples (removal model).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Indexes used for each model</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Mark-Recapture model</strong></td>
</tr>
<tr>
<td></td>
<td>$s$</td>
</tr>
<tr>
<td></td>
<td>$h$</td>
</tr>
<tr>
<td></td>
<td>$t$</td>
</tr>
<tr>
<td></td>
<td>$r$</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
</tr>
<tr>
<td></td>
<td>$i$</td>
</tr>
<tr>
<td>$M_{s,h,t,r,p}$</td>
<td>Number of marks applied</td>
</tr>
<tr>
<td>$R_{s,h,t,r,p}$</td>
<td>Number of marks recaptured</td>
</tr>
<tr>
<td>$C_{s,h,t,r,p}$</td>
<td>Catch of unmarked fish</td>
</tr>
<tr>
<td>$A_{h,t}$</td>
<td>Habitat area</td>
</tr>
<tr>
<td>$N_{s,h,t}$</td>
<td>Abundance</td>
</tr>
<tr>
<td>$\lambda_{s,h,t}$</td>
<td>Mean Abundance</td>
</tr>
<tr>
<td>$\theta_{s,h,t}$</td>
<td>Detection probability</td>
</tr>
<tr>
<td>$\alpha_{s,h}$</td>
<td>Fixed species and habitat effect for density</td>
</tr>
<tr>
<td>$\beta_{s,t}$</td>
<td>Random trip deviate on density for each species</td>
</tr>
<tr>
<td>$\gamma_{s,h}$</td>
<td>Random species and habitat effect for theta</td>
</tr>
<tr>
<td>$\delta_{s,t}$</td>
<td>Random trip deviate on theta for each species</td>
</tr>
<tr>
<td>$\tau_{s}$</td>
<td>Precision of trip deviate $\beta_2$</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Mean detection probability for the hyperdistribution</td>
</tr>
<tr>
<td></td>
<td><strong>Removal model</strong></td>
</tr>
<tr>
<td>$C_{s,t,i,p}$</td>
<td>Catch on each pass</td>
</tr>
<tr>
<td>$N_{s,t}$</td>
<td>Abundance</td>
</tr>
<tr>
<td>$\lambda_{s,t}$</td>
<td>Mean Abundance</td>
</tr>
<tr>
<td>$\theta_{s,t}$</td>
<td>Detection probability</td>
</tr>
<tr>
<td>$A_{t}$</td>
<td>Backwater area</td>
</tr>
<tr>
<td>$\eta_{s}$</td>
<td>Fixed species effect for density</td>
</tr>
<tr>
<td>$\kappa_{s,t}$</td>
<td>Random trip deviate on density for each species</td>
</tr>
<tr>
<td>$\chi_{s}$</td>
<td>Fixed species effect for theta</td>
</tr>
<tr>
<td>$\phi_{s,t}$</td>
<td>Random trip deviate on theta for each species</td>
</tr>
</tbody>
</table>
Table 3. Total available shoreline habitat sampled during 2009 and 2010. The percentage of the overall shoreline in each habitat classification is shown in parentheses. Values for 2011 are not shown and 2010 is assumed to be representative of habitat conditions in 2011 with the exception that no backwater habitat was available in 2011.

<table>
<thead>
<tr>
<th>Year</th>
<th>Sampling Month</th>
<th>Backwater</th>
<th>Cliff</th>
<th>Debris Fan</th>
<th>Sand</th>
<th>Talus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>July</td>
<td>62 (0.8 %)</td>
<td>1493 (18.5 %)</td>
<td>1959 (24.3 %)</td>
<td>1623 (20.1 %)</td>
<td>2923 (36.3 %)</td>
</tr>
<tr>
<td>2009</td>
<td>August</td>
<td>106 (1.3 %)</td>
<td>1496 (18.4 %)</td>
<td>1916 (23.5 %)</td>
<td>1661 (20.4 %)</td>
<td>2967 (36.4 %)</td>
</tr>
<tr>
<td>2009</td>
<td>September</td>
<td>138 (1.7 %)</td>
<td>1486 (18.3 %)</td>
<td>1868 (23.0 %)</td>
<td>1677 (20.6 %)</td>
<td>2967 (36.5 %)</td>
</tr>
<tr>
<td>2009</td>
<td>October</td>
<td>101 (1.2 %)</td>
<td>1486 (18.3 %)</td>
<td>1813 (22.3 %)</td>
<td>1746 (21.5 %)</td>
<td>2967 (36.6 %)</td>
</tr>
<tr>
<td>2010</td>
<td>July</td>
<td>121 (1.5 %)</td>
<td>1492 (18.2 %)</td>
<td>1972 (24.0 %)</td>
<td>1617 (19.7 %)</td>
<td>3014 (36.7 %)</td>
</tr>
<tr>
<td>2010</td>
<td>August</td>
<td>138 (1.7 %)</td>
<td>1492 (18.1 %)</td>
<td>1978 (23.9 %)</td>
<td>1663 (20.1 %)</td>
<td>2994 (36.2 %)</td>
</tr>
<tr>
<td>2010</td>
<td>September</td>
<td>74 (0.9 %)</td>
<td>1496 (18.4 %)</td>
<td>1834 (22.5 %)</td>
<td>1754 (21.5 %)</td>
<td>2994 (36.7 %)</td>
</tr>
<tr>
<td>2010</td>
<td>October</td>
<td>72 (0.9 %)</td>
<td>1496 (18.4 %)</td>
<td>1834 (22.5 %)</td>
<td>1764 (21.7 %)</td>
<td>2974 (36.5 %)</td>
</tr>
</tbody>
</table>
Figure 1. Map showing the study area and sampling reaches located on the Colorado River in Grand Canyon, Arizona. The study area is just downstream of the confluence of the Colorado and Little Colorado River. Numbers indicate kilometers downstream from Glen Canyon Dam.
Figure 2. Posterior density profiles of detection probability for each species from the removal model of fish sampled in backwaters (A). Species codes are as follows: bluehead sucker – BHS, flannelmouth sucker – FMS, humpback chub – HBC, and speckled dace – SPD. Panel B shows posterior density profiles of detection probability for the mark-recapture model of electrofishing catches. The hyperdistribution (black) is shown in comparison to profiles for each individual species and habitat type (gray).
Figure 3. Juvenile humpback chub, bluehead sucker, flannelmouth sucker, and speckled dace abundance estimates by habitat type during July, August, September, and October of 2009, 2010, and 2011. Bars represent approximate 95% credible intervals. Backwater abundance estimates (black circles) come from the removal model while abundance estimates for all other habitats (cliff, debris fan, sand, talus) come from the mark-recapture model. Generally individual month estimates for backwaters had very low uncertainty. For some
individual month, habitat, and species strata, estimates are not shown due to the absence of catches occurring in those strata. Note the lack of abundance estimates for backwater habitats in 2011 because no backwater habitat was available.
Figure 4. Total abundance trends across all habitats within the study site for juvenile humpback chub (top panel), juvenile bluehead sucker, juvenile flannelmouth, and speckled dace during sampling trips in 2009, 2010, and 2011. Abundance is presented as the relative proportion of fish estimated during each sampling trip verse the maximum abundance observed during the study for each species.
Figure 5. Mean density estimates by habitat type for juvenile humpback chub, bluehead sucker, flannelmouth sucker, and speckled dace. Solid circles are estimates from the multi-species mark-recapture model and open circles are estimates from individual species models. Bars represent approximate 95% credible intervals. Note the broken axis and truncated credible intervals for some bluehead and flannelmouth sucker density estimates.
Appendix 1: Comparison of alternative mark-recapture models with and without habitat effects.

Table A.1. Alternative mark-recapture models and criterions for assessing model fit and model selection. Modeled effects included in either the density or detection probability portions of the models are indicated with an “X”. For the abundance portion of both models, $\beta_{s,t}$ represents a random trip deviate for each species, $\alpha_{s,h}$ represents a fixed species and habitat effect, $\psi_s$ represents a fixed species effect. For the detection probability portion of the models, $\gamma_{s,h}$ is a random species and habitat effect, $\delta_{s,t}$ is a random trip deviate for each species, and $\omega_s$ represents a fixed species effect. Bayesian $p$ values ($p$) with a chi-squared discrepancy measure were used as a posterior predictive check to assess model fit. The effective number of parameters (pD), deviance information criterion (DIC), and the difference between the lowest DIC and each model ($\Delta$ DIC) are given.

<table>
<thead>
<tr>
<th>Models</th>
<th>Abundance</th>
<th>Detection Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha_{s,h}$</td>
<td>$\psi_s$</td>
</tr>
<tr>
<td>1</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Figure A.1. Comparison of mean density estimates between alternative mark-recapture models of electrofishing samples. Density estimates and 95% CRI for each fish species from model 1 (Table A1) are shown as open circles with grey bars and represent a model with no habitat effect on either detection probability or density (Combined EF). Estimates from model 2 (Table A1) are shown as black circles with black bars and represent a model with habitat effects for both detection probability and density.
Differential Predation Risk: Assessing the Role of Habitat and Turbidity in a Large River Ecosystem

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Running Title: Predation Risk in a Large River Ecosystem
Differential Predation Risk: Assessing the Role of Habitat and Turbidity in a Large River Ecosystem

Abstract

This study examined predation risk between two riverine shoreline habitats, backwater and debris fan, over three discrete turbidity levels (low, intermediate, high) to understand environmental risks associated with habitat use. While predation risk does not directly assess fitness, this information does indirectly address the consequences of using particular habitats. Earlier work in this section of the Colorado River in Grand Canyon, AZ suggests that backwater habitats are important to juvenile fish, including the federally endangered humpback chub *Gila cypha* Miller. However, it is unknown what the impacts of introduced predators may be in backwaters or other shoreline habitats. Density of two likely predators, rainbow trout *Oncorhynchus mykiss* (Walbaum) and adult humpback chub, was estimated between habitats to examine whether higher predator density was associated with patterns of predation risk. Under low and intermediate turbidity conditions, debris fan habitat showed higher relative predation risk compared to backwaters. In both habitats, the highest predation risk was observed during intermediate turbidity conditions. Density of likely predators did not significantly differ between these habitats. This information can help managers in Grand Canyon assess flow policy options designed to alter habitat availability or extant turbidity conditions.

Key words: Colorado River, *Gila cypha*, Habitat, Predation Risk, Riverine, Tethering
Fish predators can adversely affect prey populations directly through mortality (Townsend & Crowl 1991) or indirectly through modifications to prey behaviour used in foraging (Werner et al. 1983), dispersal (Townsend & Crowl 1991), and habitat selection (Mittelbach 1981; Lima 1998). Predatory interactions between nonnative and endangered species are of concern to conservation biologists (Coggins et al. 2011) because piscivory, particularly from nonnative species, can have wide ranging repercussions on recipient ecosystems (Moyle & Light 1996; Pine et al. 2007; Cucherousset & Olden 2011). For vulnerable prey like small fish, success or failure of behavioural strategies used to maximize growth and survival (Walters & Juanes 1993) are likely conditional on predator densities (Holling 1959), as well as the types of habitat selected for predator avoidance. In regulated rivers, where dams and other flow modifications have altered physical conditions including available habitats (Graf 2001; Graf 2006), differences in the relative availability of habitat selected by prey and predators alike may lead to differential predation risks. Furthermore, these physical changes are complicit since they often lead to successful establishment of nonnative species (Lozon & MacIsaac 1997; Meador et al. 2003; Johnson et al. 2008).

Throughout the Southwestern U.S., habitat degradation (Allan & Flecker 1993; Dudgeon et al. 2006) and nonnative species (Tyus & Saunders 2000; Olden & Poff 2005; Coggins et al. 2011) are implicated in the decline of native fish populations. A prime example is the Grand Canyon section of the Colorado River where physical characteristics have been altered since the construction of Glen Canyon Dam (GCD) leading to seasonally reduced flow, temperature, and sediment transport (Gloss &
Coggins 2005). Historically, the Colorado River was sediment enriched and deposited substantial quantities of sediment within and along the channel, often mantling over coarse bedrock material (cobbles and boulders) in depositional zones (Schmidt & Rubin, 1995). Structural features that act as cover to riverine species (woody debris, boulders, or turbidity) are important determinants of species distributions and may decrease predation risks and competition between individuals (Fausch 1993; Allouche 2002). In the pre-dam environment, cover in the form of structural features like boulders were likely limited in low-velocity depositional environments; whereas cover in the form of turbidity was quite common (Andrews 1991) and likely limited prey detection for visual sight feeders (Barrett et al. 1992; Yard et al. 2011). Conversely, under post-dam conditions limited sediment supply increased erosion (Rubin et al. 2002) therein exposing structural features like boulders and reduced turbidity. The changes to the physical environment are correlated in time with the introduction of nonnative rainbow trout *Oncorhynchus mykiss* (Walbaum) and the decline of the federally endangered humpback chub *Gila cypha* Miller (Gloss & Coggins 2005; Coggins et al. 2011).

Most fish habitat studies conducted in the mainstem Colorado River and tributaries have used a combination of habitat capacity models (e.g., Converse et al. 1998) and micro-habitat assessments (e.g., Gorman & Stone 1999) to establish fish-habitat associations. Although these studies have provided useful information about humpback chub, these approaches have not assessed the fitness consequences of habitat use in order to determine habitat requirements (Rosenfeld 2003; Rosenfeld & Hatfield 2006). While quantifying predation risk does not yield fitness measures, this information is useful in comparing the risks juvenile fish face in different habitats. In general,
structurally complex features (Ostrand et al. 2004; Warfe & Barmuta 2004), like vegetation or large rocky substrates, would be expected to have lower predation risk compared to structurally simple features, such as sand or bare substrates (Gadomski & Parsley 2005; Camp et al. 2011). Water clarity or turbidity levels (Gregory 1993; Allouche 2002) may also alter predation risks, especially when considering visual sight feeders like salmonids (Yard et al. 2011) that are currently the most abundant nonnative fish species in Grand Canyon (Coggins et al. 2011). In general, increased turbidity levels are thought to reduce foraging success of predators and thus decrease the direct and indirect effects of predation (Barrett et al. 1992; Carter et al. 2010).

Our study objective was to quantify relative predation risks with respect to shoreline habitats and turbidity conditions. Information on predation risk will help assess habitat requirements for juvenile native fish in Grand Canyon and inform management actions. Our hypotheses were (1) that predation risk was greater in shoreline features lacking structural complexity; and (2) that predation risk was greater under lower levels of turbidity because visual predators may be more efficient. To test these hypotheses, we evaluated two independent variables: shoreline habitat (debris fans and backwaters), and three turbidity levels (low, intermediate, and high). Additionally, we estimated habitat specific densities for two potential piscivores, adult rainbow trout and humpback chub (Stone & Gorman 2006; Yard et al. 2011), to determine if predator densities correspond with predation risks.
Methods

Study Area and Habitat Descriptions

The study area is located downstream from Lees Ferry on the Colorado River in Grand Canyon, Arizona (Figure 1), and contains the largest population of humpback chub in the Colorado River. Approximately 95% of the population is found in close proximity (± 10 km) to the Little Colorado River (LCR) and its confluence with the Colorado River (Valdez & Ryel 1995; Gorman & Stone 1999). The LCR is a seasonally warm unregulated tributary where humpback chub successfully spawn, juveniles rear, and disperse into the Colorado River (Robinson et al. 1998; Gorman & Stone 1999). This tributary and others found further upstream periodically flood and supply suspended sediment to the Colorado River (Topping et al. 2000). Although current sediment transport levels are less than the pre-dam environment, these tributaries frequently flood creating periods of high turbidity in the Colorado River. Furthermore, juvenile chub, which disperse from the LCR, often occupy different types of shoreline habitat (Converse et al. 1998; Gerig 2012); some of which are located below hydraulic constrictions (riffles and rapids) formed by debris fans.

Geomorphically, debris fans are one of the most fundamental and repetitive units found throughout the Colorado River (Schmidt & Rubin 1995) that actively control the types and proportions of physical and hydraulic features used by the fish community. Debris fans constrict river flow forming rapids, riffles, glides, and create low velocity regions upstream of these hydrologic features. Below the constriction formed by debris fans, the channel width expands and creates large recirculation zones or eddies (Schmidt & Graf 1990) where fine sediment is often deposited (Figure 2). Two types of
depositional features occur (1) separation bars (structurally complex areas comprised of unsorted rocky and sandy material, moderately deep, and reduced flow velocity), and (2) backwaters (areas lacking cover, comprised of homogeneous sandy substrate, shallow, and minimal flow exchange). The sites chosen to assess predation risk were representative of separation bars (hereafter called debris fan, mixed substrate of small to large boulders and intermittent sand, ≈0.02-m/sec surface velocity, and ≈75° bank slope) and backwaters (eddy return channel dominated by sand substrate, very low surface velocity, and ≈ 5° bank slope). Backwater habitat has been broadly defined in Grand Canyon, but this study considers only areas formed by eddy return channels described in Schmidt (1990) and not backwaters formed by channel margin sand deposits. Both the altered flow and sediment transport regimes have changed the type of sandy shoreline features found in Grand Canyon (Rubin et al. 2002), making fine sediment deposits less extensive (Schmidt & Rubin 1995) and likely increasing the proportion of more structurally complex shoreline types. Shorelines associated with debris fans (Converse et al. 1998) and backwaters (Grams et al. 2010) have some of the higher sub-adult humpback chub catches and no study has yet considered the predation risks of smaller fish associated with these shoreline habitats.

River turbidity, reported as formazin nephelometric units (FNU) (Voichick & Topping 2010) was continuously monitored (15 minute intervals) using acoustic-Doppler profilers (Topping et al. 2007) at USGS gaging station 09402500. Because the study site was located 38 km upstream from the actual gage station, data were adjusted to account for the difference in travel time between sites using an unsteady flow discharge model (Wiele & Griffin 1997). During predation risk experiments, turbidity values were
averaged over each tethering trial and each trial was assigned to a discrete level (low
[<30 FTU], intermediate [>31-600 FTU], high [>600 FTU], Table 1). For the predator
density estimates, turbidity was used as a covariate and averaged from 1800 to 2359
hours during each sampling event (see below).

Predation Risk

Tethering experiments were used during September and October 2010 to quantify
predation risk, defined as the binomial proportion of predation events (successes)
observed for a single trial of 10 tethered fish conducted for a 1-2 hour period.
Successes were defined as missing or partially consumed fish. Fathead minnows
_Pimephales promelas_ Rafinesque, (40-70-mm total length) were tethered to a ganion
using 0.5-m of braided fishing line (4.5-kg test, 0.15-mm diameter) passed through the
caudal peduncle above the spine and ventrally knotted. For each habitat, a set of
ganions were then anchored to a lead line (6.4-mm dia.) using clips attached at 1-m
intervals deployed parallel to the shore at depths of 50 to 130-cm. Paired trials were
conducted in each shoreline habitat (debris fan and backwater) across three discrete
turbidity levels. Trials were run continuously during two or three day periods each
month. Additionally, a control was established using an enclosure and ran concurrent
with the predation risk trials to estimate the loss rate of tethered fish independent of
predators. The enclosure consisted of hardware cloth (30-mm mesh) anchored into the
substrate where all fish were removed prior to deploying tethered fish. The enclosure
was located in backwater habitat during the September trials (generally low and high
turbidity) and in the debris fan habitat during the October trials (generally intermediate
turbidity).
We used a mixed effects logistic regression approach in program R (lme4 package, R Development Core Team 2012) to compare alternative models of predation risk for different habitat conditions (shoreline type, turbidity level, and day vs. night). A random effect for each sampling event was added to account for overdispersion (mean deviance ratio > 1). Models were assessed using Akaike’s information criterion (AIC) with a correction for small sample size and the highest weighted model (lowest AIC) was selected (Burnham & Anderson 2002). Predation risk for each habitat type and turbidity level was estimated and 95% confidence intervals were determined using a non-parametric bootstrapping procedure (10,000 iterations, with replacement). Pairwise Wilcoxon rank-sum tests with a Bonferroni correction were used to test for significant differences (alpha level = 0.05) in predation risk between shoreline types and turbidity levels.

**Predator Densities**

Electrofishing boats were used to sample the fish community in shoreline areas repeatedly during July, August, September, and October 2010. Three sites, each approximately 1.2-km in length, were repeatedly sampled three times each month with 24 hours between passes. Electrical current was held to 5,000 W of power (15-20 amps and 200-300 volts) using variable-voltage pulsators (CPS Mark XXII™). Sampled shorelines were subdivided into approximately 200-m sections of continuous habitat and classified as one of four discrete shoreline types (cliff, sand deposits, debris fan, or talus). A total of approximately 7,800-m of linear shoreline was sampled each trip. Because sandy shorelines containing backwaters were often too shallow to access with boats, this habitat type could not be directly sampled by electrofishing. For analytical
purposes we assumed that electrofishing catches for backwaters were similar to shoreline sections classified as sand deposits.

Predator densities for humpback chub (>180-mm total length) and rainbow trout (>200-mm total length) were estimated using N-mixture models (Royle 2004) from the spatially and temporally replicated electrofishing catches during July-October 2010. Poisson or negative binomial models were compared to account for possible overdispersion in the catch data (Kery et al. 2005; Joseph et al. 2009). Detection probability (p) was assumed to be binomially distributed and estimated using the repeated counts within a continuous section of habitat. Using either a log or logit link function, predator densities were estimated in relation to covariates that influenced their distribution and detection, these included shoreline habitat type, linear distance sampled, sampling month, and turbidity. All numerical covariates were transformed into standard normal deviates prior to modeling.

Model comparisons were made using AIC and the most parsimonious model for each species was chosen. Models were fit using integrated likelihood methods with the UNMARKED package in program R (Fiske & Chandler 2011; R Development Core Team 2012). Using a parametric bootstrapping procedure that evaluated fit statistics (Pearson’s chi-squared statistic and sum-of-squared error, Fiske & Chandler 2011), we assessed the fit of most highly supported model for each species. Uncertainty of model coefficients and estimates were assessed by extracting the square root of the diagonal of the covariance matrix and an asymptotic normal approximation was used to assign 95% confidence intervals. Estimated mean density and detection for humpback chub...
and rainbow trout was accomplished using the most highly supported model for each species, catch information, and covariates for September and October 2010.

Results

Predation risk under low and intermediate turbidity levels was higher in debris fan compared to backwater habitat, and also highest in both habitats under intermediate turbidity (Figure 3). Based on AIC comparisons of alternative logistic regression models, the highest weighted model allowed predation risk to vary by both turbidity and habitat (Table 2), indicating that shoreline type and turbidity are important variables explaining predation risk in this study. The most highly supported model did not include the covariate for day versus night. Pairwise Wilcoxon rank-sum tests confirmed significant differences (significance level = 0.05) between habitats during low and intermediate turbidity conditions (Table 3, Figure 3). During low turbidity, predation risk was higher in the debris fan (35.0%) compared to the backwater (5.0%) habitat. Predation risk under intermediate levels of turbidity increased in both the debris fan (73.0%) and backwater (45.7%) habitat. Under high turbidity there was no significant difference in risk between debris fan (7.0%) and backwater (11.7%). Results for the control (enclosure) show that the frequency of false positives (fish missing from the tether absent any predators) was unobserved in the backwater (0.0%) and very low in the debris fan (0.0086%). The control results showed minimal loss of tethered fish and suggest that our metric is an unbiased estimate of predation risk.

Based on AIC comparisons, the most highly supported Poisson N-mixture model for rainbow trout and humpback chub contained sampling month, shoreline habitat type,
and turbidity as covariates for detection and sampling month and shoreline habitat type
as covariates for density. There was substantial model-selection uncertainty with < 4
delta AIC units separating the top ten models for both species (Table 4). Overall, the
most highly supported model for rainbow trout (lowest AIC) was the null negative
binomial model that did not support covariates of detection or density. Across all models
considered, there was considerable uncertainty and the data did not provide sufficient
information for clear separation of one model being highly supported (Table 4). The
most highly supported Poisson models for each species are presented in order to make
consistent comparisons between species and to present inferences from a model that
contains covariates thought to be important to both detection and density.

Density estimates for rainbow trout and humpback chub were similar between
months and shoreline habitats (Figure 4). Confidence intervals for humpback chub
density during September were wide, but despite this uncertainty, point estimates in
debris fan are higher than in sand deposits. Under the most highly supported rainbow
tROUT covariate model, estimated density was lower in debris fan compared with sand
deposits although confidence intervals overlapped. In general, no large differences in
predator densities were observed between shoreline habitats, time periods, or fish
species. Estimates of detection for humpback chub were lower and more precise
compared to rainbow trout. Although detection probabilities for rainbow trout were
higher in debris fans than sand deposits, these differences were not significant.
This study observed higher predation risk in debris fan habitat (typified by higher complexity and increased depth), and higher risk under moderately elevated turbidity levels. Both results appear counter to previous work, where cover in the form of structural complexity (e.g., vegetation and crevices) or turbidity are factors often recognized as reducing predation risk to small sized fish (Crowder & Cooper 1982; Werner et al. 1983; Werner & Hall 1988). However, most research on predation risks and structural complexity has occurred in lentic or near-shore marine systems, often considering vegetated habitats (Crowder & Cooper 1982; Gorman et al. 2009; Nanjo et al. 2011). Predation risks have been examined in some lotic systems (White & Harvey 2001; Camp et al. 2011), yet more research in large canyon bound rivers with predominately rocky substrates is warranted to further determine the role of structural complexity for small fish. At first glance, our findings relative to cover seem somewhat counterintuitive, particularly when we only consider cost-benefit trade-offs of prey items without taking into account similar trade-offs governing larger aquatic piscivores (Grol et al. 2011; Ahrens et al. 2012). Behavioural responses and habitat avoidance have been experimentally observed for large fish exposed to terrestrial or avian piscivores (wading/diving animals) in shallow clear waters (Power et al. 1989; Crowder et al. 1997) and these responses may help explain the observed patterns of predation risk.

Typically, larger fish frequently demonstrate piscivorous behaviour, but these same fish are not without real or perceived risk from predation themselves. Numerous avian piscivores such as the common merganzer *Mergus merganser*, great blue heron *Ardea herodias*, bald eagle *Haliaeetus leucocephalus*, and osprey *Pandion haliaetus*
are found in Grand Canyon (Carothers & Brown 1991), and are known to successfully forage on nonnative and native fish, particularly rainbow trout and humpback chub (Brown & Trosset 1989; Wasowicz & Yard 1993). Although cover is more commonly thought of as a feature that reduces predation risk to small or juvenile fish prey (Gregory 1993; Carter et al. 2010), cover in the form of depth or turbidity may also benefit larger fish (Harvey & Stewart 1991; Yard et al. 2011) by reducing additional risk from avian predators (Lonzarich & Quinn 1995; Reinhardt & Healey 1997). Ontogenetic differences found among fish have been shown to influence the types of habitats selected for both foraging and refuge (Reinhardt & Healey 1997; Korman & Campana 2009) in response to predation risk (Quinn & Peterson 1996). Therefore, greater and more heterogeneous depths may provide cover for piscivores by making them less discernible to both fish prey as well as avian predators (Allouche 2002). Several studies have experimentally shown that predation risk is high for large fish in shallow clear water systems (Harvey & Stewart 1991; Lonzarich & Quinn 1995; Allouche & Gaudin 2001). Similarly, we observed that under conditions of high water clarity that shallow backwaters (< 1.5 m) lacking any form of structure had significantly less predation risk to small fish than structurally complex habitats, like debris fans, that were considerably deeper.

In general, rainbow trout (MacCrimmon & Kwain 1969; Kwain & McCauley 1978) and humpback chub (Valdez & Ryle 1995; Stone & Gorman 2006) demonstrate negative phototaxic responses. Visual acuity is known to play an important role in foraging behaviour of rainbow trout (Barrett et al. 1992; Al Shaw & Richardson 2001), while for humpback chub, foraging ecology is less understood. Adult humpback chub have been documented to be opportunistically piscivorous and move inshore at night to
feed (Stone & Gorman 2006). Deductively, humpback chub are more likely to be effective at foraging under turbid conditions since this species evolved in a highly variable turbid environment (Stone 2010). Visual sight feeders, like rainbow trout, are less effective at foraging under increased turbidity, because of decreasing reactive distance (Barrett et al. 1992; Huenemann et al. 2012) that often leads to changes in feeding behaviour (Berg & Northcote 1985; Gregory & Northcote 1993) and habitat use (Miner & Stein 1996). Similarly, telemetry studies of humpback chub have shown that during elevated turbidity fish moved from deeper sections of the channel (primarily large eddies) into shallow backwaters and rocky shorelines (Valdez & Ryle 1995; Gerig 2012).

Inshore movements in response to turbidity by adult humpback chub, and likely by rainbow trout as well, may partially explain why the highest predation risk occurred at intermediate levels of turbidity. In contrast, the highest levels of turbidity tested were sufficiently high and long enough to have caused considerable physiological stress (osmotic and respiratory) to fish. Rainbow trout specifically are likely to become inefficient at foraging during higher turbidity levels (Sigler et al. 1984, Redding et al. 1987). Newcombe & Macdonald’s (1991) literature review on suspended sediment effects, suggests that the highest range in turbidity we observed would seemingly have caused reduced foraging activities for rainbow trout (Berg & Northcote 1985; Gregory & Northcote 1993). It is less clear how turbidity would have influenced humpback chub because of its evolutionary history in highly turbid environments. Stone (2010) suggested that humpback chub’s visual capabilities are probably suppressed at high turbidity levels, but other sensory abilities are used to detect prey.
In Grand Canyon, both rainbow trout and humpback chub are recognized for having high diet overlap and appear to be opportunistically piscivorous (Valdez & Ryle 1995; Donner 2011), as well as being able to capture fish prey under turbid conditions (Stone & Gorman 2006; Yard et al. 2011). Therefore, one should expect that predation risk would vary according to differences in prey and predator densities, particularly since the functional response of a predator to its prey (Holling 1959) is often modified by the types of prey and predator densities (Kratina et al. 2009). Our estimates for predator densities (approximately 20 fish / 250-m) for both rainbow trout and humpback chub did not vary over this study period or between habitats. Also, rainbow trout densities below the LCR still remain less than when trout densities (approximately 70 fish / 250-m; Coggins 2008) were numerically suppressed in 2003 for experimental purposes to conserve native fish (Coggins et al. 2011; Yard et al. 2011). We found that predation risks, presumably from rainbow trout or humpback chub, were indistinguishable from each other since each of the two species had comparable densities, and that these two species were distributed similarly between debris fans and sand deposits. No sampling of backwater areas for potential fish predators was feasible, but estimated densities from habitats dominated by sand deposits offer a similar comparison. It remains uncertain which of the two fish species was more or less responsible for the observed predation risk. It is likely that predation risk will differ depending on predator density and availability of fish prey.

The use of tethering as a measure of predation risk has received criticism (Peterson & Black 1994) and much subsequent debate (Aronson & Heck 1995; Aronson et al. 2001) centered on its equivalence as a proxy to natural predation rates (Peterson
& Black 1994; Kneib & Scheele 2000; Linehan et al. 2001). Often the greatest concern is the potential for methodological artefacts to bias possible inferences (Peterson & Black 1994; Curran & Able 1998; Kneib & Scheele 2000). Tethering inhibits prey from escaping and yields a relative, not absolute measure of predation, which can be compared across habitats or environmental conditions (White & Harvey 2001; Laplante-Albert et al. 2010; Camp et al. 2011). While tethering does not quantify absolute mortality, the technique has been widely applied to study predation risk under the assumption that methodological artefacts do not co-vary among habitats (Linehan et al. 2001). In this study, a specific concern was the possibility of tethered fish tangling and escaping in the rocky substrate, thus producing an artefact of the tethering that would co-vary among habitats. The control demonstrated very low loss of tethered fish in either habitat and suggests that the metric is an unbiased estimate of predation risk.

Examining predation risks that juvenile fish face in this section of the Colorado River has implications for native fish conservation, especially with respect to juvenile humpback chub that spawn in the LCR and disperse into mainstem shoreline habitats (Valdez & Ryel 1995; Robinson et al. 1998). The Colorado River is subject to management actions such as experimental high flows that conserve sediment via creating sandbars and produce aquatic habitats such as backwaters (Melis 2011). Many fish species, including juvenile humpback chub, show positive habitat selection for backwaters because they are warm and shallow (Dodrill 2012); yet, this habitat is not a common feature found in Grand Canyon (Goeking et al. 2003). Near the confluence of the Colorado River and LCR, backwaters support a small proportion of the overall fish abundance (Dodrill 2012), partly because habitat availability is limited by the quantity of
depositional environments (Schmidt 1990), sediment supply (Topping et al. 2000), and antecedent flow conditions (Topping et al. 2005). This study provides inferences on predation risk between habitats and although absolute fitness measures of backwater habitat use are uncertain, informs managers of the consequences of habitat use decisions for juvenile native fish.

Our results are useful in understanding the inherent ecological risks associated with using particular habitats. This is particularly relevant in this segment of the Colorado River below the LCR confluence where management actions are often designed to improve conditions for native fish populations. Understanding the factors that affect predation risk for juvenile native fish facilitates a mechanistic understanding of how species may respond to future management actions directed at manipulating habitats, physical conditions, or nonnative predators. The effects of predation risk on prey can be manifest as behavioural (Lima 1998), habitat use (Creel et al. 2005), or foraging responses (Walters & Juanes 1993) that ultimately can result in reduced survival, growth, or reproduction (Creel et al. 2007). Some research suggests that the effects of predation risk on prey demographics can be as large as the direct effects of predation (Nelson et al. 2004; Preisser et al. 2005). Selecting rearing locations requires fish to assess trade-offs between acquiring food resources and avoiding predation (Grol et al. 2011; Ahrens et al. 2012). Juvenile humpback chub that emigrate from the LCR to the Colorado River must choose between shoreline habitats with varying resources and risks. Understanding these trade-offs will ultimately benefit the management of native fish in the Colorado River.
Acknowledgments

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Fausch K.D. (1993) Experimental analysis of microhabitat selection by juvenile steelhead (Oncorhynchus mykiss) and coho salmon (O. kisutch) in a British
Columbia stream. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 1198-1207.


Table 1. Summary of continuous turbidity measurements shown in formazin turbidity units (FTU) during each of the turbidity conditions. The number of trials for each turbidity level and habitat are shown.

<table>
<thead>
<tr>
<th>Turbidity</th>
<th>Mean FTU</th>
<th>SD</th>
<th>Range</th>
<th>Backwater Trials</th>
<th>Debris Fan Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>16</td>
<td>7</td>
<td>6 – 28</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Intermediate</td>
<td>165</td>
<td>191</td>
<td>34 – 594</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>High</td>
<td>1507</td>
<td>1065</td>
<td>606 – 4830</td>
<td>24</td>
<td>23</td>
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</table>
Table 2. Model selection of mixed logistic regression models describing tethered fish mortality. Labels in parentheses represent factors by which mortality could vary including: Habitat – backwater or debris fan, Turbidity – low, intermediate, high, Time – day or night, or dot (.) – constant mortality. The column headings K, AICc, and ∆ AICc denote the number of model parameters, Akaike’s information criterion corrected for small sample sizes, and the difference between the smallest AICc considered and the current model.

<table>
<thead>
<tr>
<th>Model</th>
<th>K</th>
<th>AICc</th>
<th>∆ AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>M(Habitat X Turbidity)</td>
<td>6</td>
<td>338.5</td>
<td>0.0</td>
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<tr>
<td>M(Time + Turbidity)</td>
<td>4</td>
<td>367.0</td>
<td>28.4</td>
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<tr>
<td>M(Turbidity)</td>
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<td>367.6</td>
<td>29.0</td>
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<tr>
<td>M(Time + Habitat)</td>
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<td>386.6</td>
<td>48.1</td>
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<tr>
<td>M(Habitat)</td>
<td>2</td>
<td>389.6</td>
<td>51.0</td>
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<tr>
<td>M(Time)</td>
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<td>398.4</td>
<td>59.9</td>
</tr>
<tr>
<td>M(.)</td>
<td>1</td>
<td>401.1</td>
<td>62.6</td>
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</table>
Table 3. Pairwise comparisons treatments using Wilcoxon rank-sum tests with Bonferroni correction applied (α = 0.05 level of significance).

<table>
<thead>
<tr>
<th></th>
<th>Backwater</th>
<th>Backwater</th>
<th>Backwater</th>
<th>Debris Fan</th>
<th>Debris Fan</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
<td>Intermediate</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Backwater Low</td>
<td>1</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Backwater</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Intermediate</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Debris Fan High</td>
<td>1</td>
<td>1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Debris Fan Low</td>
<td>0.011</td>
<td>&lt;0.001</td>
<td>1</td>
<td>&lt;0.001</td>
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<tr>
<td>Debris Fan</td>
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<td>0.003</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Intermediate</td>
<td></td>
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<td></td>
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<td></td>
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</tbody>
</table>
Table 4. Model selection of N-mixture models including covariates of both detection (p) and abundance (N). The top ten Poisson models for both adult humpback chub and adult rainbow trout are shown. Covariates of detection and abundance include sampling month (MONTH), shoreline habitat (SHORE), linear shoreline sampled (DIST), and turbidity (FTU). The dot (.) represents constant detection or abundance. The column headings K, AIC, and Δ AIC denote the number of model parameters, Akaike’s information criterion, and the difference between the smallest AIC considered and the current model. The abbreviation HBC stands for adult humpback chub, and RBT stands for adult rainbow trout.

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>K</th>
<th>AIC</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBC</td>
<td>p(MONTH + SHORE + FTU)N(MONTH + SHORE)</td>
<td>7</td>
<td>203.68</td>
<td>0</td>
</tr>
<tr>
<td>HBC</td>
<td>p(MONTH + FTU)N(MONTH)</td>
<td>5</td>
<td>203.88</td>
<td>0.20</td>
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<tr>
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Figure 1. Map showing the Colorado River in Marble and Grand Canyons, Arizona. The study area is located just downstream of the confluence of the Colorado and Little Colorado Rivers. Numbers indicate km downstream of Glen Canyon Dam.

Figure 2. Schematic showing the relative location of the backwater and debris fan habitats used in tethering experiments (white boxes). Contours (1-m intervals) represent the depths relative to the water level (bold black line).

Figure 3. Predation risk expressed as percent mortality of tethered fish in each shoreline habitat type and under each turbidity level. Bars represent upper and lower 95% confidence intervals.

Figure 4. Predicted mean density (fish per 100-m) and detection for adult humpback chub (A, C) and rainbow trout (B, D) during September and October, 2010. Bars represent 95% confidence intervals.
Figure 1.
Figure 2.
Figure 3.

% Mortality of Tethered Fish (+-95% CI)

Low          Intermediate          High

Turbidity

Backwater    Debris Fan
Figure 4.
Assessing juvenile fish population demographic responses to a steady flow experiment in a highly regulated large river ecosystem: A test in the Colorado River below Glen Canyon Dam

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Abstract

The Colorado River below Glen Canyon Dam, Arizona, is the focus of the Glen Canyon Dam Adaptive Management Program—a diverse group of managers and stakeholders tasked with reducing uncertainty associated with flow policies from Glen Canyon Dam on the natural, recreational, and cultural resources located in downstream river reaches. One critical component of the adaptive management program is the population viability of the federally endangered humpback chub *Gila cypha* and how dam operations and other management actions influence population dynamics of this species, particularly juvenile life stages. In this paper we provide the first direct assessment of juvenile humpback chub apparent survival rates (1- mortality-emigration) and abundance in the Colorado River below Glen Canyon Dam. We conducted the assessment over 3 years (July 2009 – July 2012) within a larger study of juvenile fish populations in response to an experimental steady discharge from Glen Canyon Dam. Our most highly supported model estimated different annual apparent survival rates of humpback chub (generally 40-150 mm TL) for each study year (69, 46, and 30% for 2009, 2010, and 2011, respectively). This model provided limited support for flow effects on survival. Effects of the steady flow experiment were relatively small in comparison to annual variation in juvenile apparent survival. Closed and open population methods were also used to estimate juvenile humpback chub (generally <100-mm TL) abundance in the ~4 km river study reach. Abundance estimates for the NSE study reach during 2009-2011 from closed population models ranged from 338-4,042 individuals, while abundance from open models ranges from 1537-7,006, with all models estimating increases in juvenile humpback chub abundance through time. Whether these annual changes in apparent survival and abundance are reflective of broader trends or simply inter-annual variation is not known. One key result that we demonstrated through simulation is that our methodological framework is likely adequate to provide reasonable apparent survival and abundance estimates for juvenile humpback chub. Our work also demonstrates that these fish are rearing in the mainstem Colorado River, a habitat formerly considered a sink for juveniles. These are significant findings and demonstrate how new field efforts can inform operations of one of the most important dams in the US as well as aid in managing endangered fish populations in regulated rivers worldwide.

Optimizing dam operations to minimize physical and ecological impacts to associated ecosystems while still meeting human resource needs is a challenging goal (Anderson and Woosely 2005, Richter and Thomas 2007). For instance, the Colorado River in the western United States is identified as the “…most legislated, most debated, and most litigated river in the entire world. It has more people, more industry, and a more significant economy dependent on it than any comparable river in the world” (Reisner 1986). This riverine ecosystem is highly altered due to flow modifications from numerous dams and water diversion projects. The 1995 Environmental Impact Statement (EIS) on Glen Canyon Dam in Grand Canyon National Park identified key uncertainties for the Colorado River related to water releases from the dam (http://www.usbr.gov/uc/rm/amp/background.html). In an attempt “…to provide an organization and process for cooperative integration of dam operations, downstream resource protection and management, and monitoring and research information…” (USBR 2013, http://www.usbr.gov/uc/rm/amp/) in the UNESCO recognized
reach in Grand Canyon National Park, the Glen Canyon Dam Adaptive Management Program (GCDAMP) was developed in the late 1990’s following the 1995 EIS. The GCDAMP creates a unique regulatory and operational opportunity in the Grand Canyon reach of the Colorado River to experimentally assess the impacts of various flow policies on downstream resources on a system-wide basis. Experiments can identify policy choices to manage the system for specific natural or ecological features (i.e., sandbar conservation for recreation, native fish restoration, recreational trout fishing, etc.) while meeting power and water needs of human users. As an example, if experiments could identify flow policies that simultaneously satisfy legal, ecological, cultural, recreational, municipal, and industrial constraints of the Colorado River, this information would be invaluable to managers of Glen Canyon Dam. Among the least flexible constraints considered in GCDAMP policies is the application of the US Endangered Species Act in conserving endangered species (e.g., humpback chub, Kanab ambersnail *Oxyloma haydeni kanabensis*) without precluding other benefits and uses of the Colorado River and associated reservoirs.

One of the most important drivers of GCDAMP is population recovery of humpback chub (*Gila cypha*) a large-bodied, morphologically distinct minnow endemic to the Colorado River basin (for additional references see Goulet and LaGory 2009). Humpback chub are currently listed as endangered under the Endangered Species Act. The reason for declines in humpback chub populations in Grand Canyon are unknown but likely include: (1) negative interactions with non-native fish (Coggins et al. 2011; Yard et al. 2011), (2) loss of essential habitats due to flow, temperature, and sediment input modifications (Converse et al. 1998; Clarkson and Childs 2000; Stone and Gorman 2006), and (3) non-native parasites (Minckley 1991, Valdez and Ryel 1997).

Most remaining humpback chub occupy the Colorado River near the confluence with the Little Colorado River, the largest tributary within Grand Canyon (Figure 1). These adults participate in a spawning migration from the mainstem Colorado River to the Little Colorado River after which the majority of adults return to the mainstem Colorado River (Kaeding and Zimmerman 1983, Valdez and Ryel 1997, Gorman and Stone 1999). Juvenile humpback chub may then emigrate to the mainstem Colorado River as larvae or small juveniles. Their survival
in the highly modified Colorado River is potentially low because of cold-water temperatures and negative interactions with non-native species (Clarkson and Childs 2000; Yard et al. 2011). Juvenile humpback chub may also rear in the Little Colorado River for several years where warmer water temperatures and lower predation risk from non-native species (Kaeding and Zimmerman 1983; Clarkson and Childs 2000; Gorman and Stone 1999; Limburg et al. in-review) may lead to higher juvenile survival before they join the adult spawning migration (Gorman and Stone 1999).

Recent experimental policies implemented by GCDAMP intended to benefit the population of humpback chub include non-native predator removal (Coggins et al. 2011, Yard et al. 2011), fluctuating flow mitigation (Ralston 2011), and habitat restoration (Melis et al. 2011). A critical objective of these experiments is enhancing vital rates of juvenile humpback chub (USDOI 2008) to increase recruitment to the adult population. However, interpreting effects of these ecosystem experiments on juvenile humpback chub is challenging due to the difficulty of directly monitoring juveniles. Trends in juvenile humpback chub have traditionally been made through inferences based on catch-rate indices as well as from reconstructions of recruitment patterns from recaptures of tagged adult humpback chub as part of the standard adult humpback chub assessment program (Age-structured mark-recapture, ASMR, Coggins et al. 2006a; Coggins et al. 2006b). Due to uncertainty in the age assignment when fish are first tagged, birth-year may be misassigned resulting in a reduction in the annual variability of reconstructed recruitment trends (Coggins and Walters 2009). While the ASMR methods provides robust estimates of recruitment trends, GCDAMP members, resource managers, and dam operators have sought complimentary approaches to assess juvenile fish population with reduced lag time between a management action and inferences of cohort strength.

Current operations at Glen Canyon Dam follow electrical power demand with diel fluctuations in dam discharge, often referred to as hydropeaking (Figure 2). These water level fluctuations can cause shifts in the availability of different nearshore habitat types depending on shoreline bank angle (Korman and Campana 2009). Littoral areas near the dam experience larger changes in habitat availability than steep-angle shorelines located further downstream. These water level fluctuations can have significant ecological effects on juvenile fish. For
example, transitory availability of habitat due to hydropeaking often forces juvenile fish away from preferred habitats (Bunt et al. 1999) leading to slower growth rates (Korman and Campana 2009) and potentially exposing fish to higher predation rates by altering foraging arenas (Walters and Juanes 1993; Ahrens et al. 2012). In shallow littoral habitats (especially vegetated cobble bars or backwaters), warm water microhabitats can develop under steady flow conditions (Korman and Campana 2009, Ralston 2011), which may be important to juvenile life stages of warm water native fish (Trammel et al. 2002, USDOI 2008, Ralston 2011).

Due to concern over the potential ecological effects of fluctuating flows, experimental steady flows (i.e., constant water release from Glen Canyon Dam) were implemented by GCDAMP during September and October 2008-2012 (Figures 2 and 3) to assess response of juvenile humpback chub populations in terms of habitat use, growth, or survival. The goal of these steady flow experiments was to improve recruitment and population growth rate of humpback chub over the extant fluctuating flow policy. While a steady flow policy is intuitive by design, the benefits to native fish are highly uncertain and expensive to implement in terms of lost power revenue (Palmer et al. 2004, Ralston 2011).

In this paper we (1) develop a capture-recapture framework to directly estimate abundance and survival of juvenile humpback chub less than 200-mm TL, and (2) assess trends in humpback chub survival and abundance in years with experimental steady flows (2009-2011). We present field techniques and analytical approaches used for this work and then discuss how our results fit within the broader context of understanding juvenile humpback chub population dynamics in Grand Canyon. If juvenile humpback chub survival rates improved during experimental steady flows, then steady flows could be considered as part of the long-term management plan for Glen Canyon Dam operations. Careful consideration of how this policy may affect other Colorado River resources downstream of Glen Canyon Dam (i.e. rainbow trout, sandbars) as well as meeting power and water delivery contracts is necessary. Understanding the fish-flow relationship is increasingly important globally in order for dam operations to mitigate negative ecological changes.
**Methods**

There are nine aggregations of humpback chub found in the Colorado River downstream of Glen Canyon Dam (Valdez and Ryel 1997). The lower 14 km of the Little Colorado River (LCR) and the associated inflow reach of the Colorado River support the largest known humpback chub population anywhere in the Colorado River basin (Valdez and Ryel 1997, Coggins et al. 2006a, Figure 1). Unlike the other identified Colorado River aggregations, this location supports a functioning population where all life stages and successful reproduction can be routinely observed. The Little Colorado River (LCR) has a spring-sourced base flow of about 6.2 m$^3$/sec and large seasonal fluctuations in flow based on winter runoff and summer monsoon rains. Water temperatures generally range from 10-25° C, which is similar to the Colorado River in winter but much warmer during summer due to hypolimnetic water releases from Glen Canyon Dam (Figure 4). Although the LCR flow and temperature are not influenced by Glen Canyon dam operations, the mainstem-resident juvenile humpback chub are the targeted benefactors of GCDAMP experimental policies because of their migration patterns between LCR and the regulated Colorado River.

We sampled the Colorado River for juvenile humpback chub from river kilometer (rkm) 102 to rkm 106 a total of 14 times from 2009-2012. Sampling included 12 consecutive days during July, August, September, and October of 2009-2011 as part of a larger cooperative Nearshore Ecology (NSE) research project and to complete our survival and abundance estimation we included nine consecutive days of sampling in April and July 2012 from rkm 102 to rkm 104.5. We used two gear types to sample the fish community: un-baited mini hoopnets (50 cm diameter, 100 cm long, single 10-cm throat, made of 6-mm nylon mesh, fished over 24-hour intervals) and boat electrofishing (pulsed DC current at 15-20 amps, 200-300 volts, 7-10 seconds per meter of shoreline, repeated 24 to 72 hours apart for three passes per trip). Initial sampling in July 2009 included 48 hoopnets in the upper 1.3 km of the sampling universe, which expanded to 60 hoopnets in August-October, all of which were fished 12 consecutive days for about 24 hours each. In 2010 and 2011 we further expanded hoopnet efforts to include 12 passes of 80 hoopnets over the upper 2.5 km sampling reach. In 2012 hoopnet sampling was further increased to 90 hoops in the same 2.5 km, but with only seven, 24-hour passes. All
available shoreline was electrofished during the twelve sampling trips in 2009-2011 following guidelines in Korman and Campana (2009), to target small fish with an emphasis on maintaining slow boat speeds of 5-10 sec/m of shoreline. Electrofishing effort in 2012 was reduced to the upper 2.5 km of the sampling universe.

Following capture, we measured, tagged, and then released humpback chub to the location where they were captured. Individual humpback chub between 40 and 100 mm total length (TL) were given batch marks using visible implant elastomer (VIE, Northwest Marine Technologies). These elastomer batch marks were identifiable to individual sampling trip and gear. On subsequent recapture, if the humpback chub was still <100 mm TL, it was given another VIE (batch) mark coded for that respective sampling trip and gear. Any humpback chub >100 mm TL received a 134.2 kHz passive integrated transponder (PIT) tag (9-mm long, BIOMARK) with a unique number identifiable to individual fish. Data for these analyses include VIE-marked humpback chub (40-99 mm TL) until the first time they were captured above 100 mm TL, at which point they received a PIT tag, were released, and considered removed from the study population.

Using simulation to evaluate model accuracy

In capture-recapture experiments it is possible to confound apparent survival rates and capture probability estimates due to the inability to distinguish between fish that died (low survival) and fish that lived but were not observed (high survival, low capture probability). We expected juvenile humpback chub capture probabilities to be low based on pilot samples and other capture-recapture experiments in large rivers (Coggins et al. 2006a; Lauretta et al. 2013). These low capture probabilities combined with short discrete sampling intervals (designed around the flow experiment) could lead to erroneous survival estimates. To assess this we generated data sets (program GENCAPH1; http://www.mbr-pwrc.usgs.gov/software/gencaph1.shtml) with known survival rates, capture probabilities, and sampling intervals similar to our expected values based on our pilot study and experimental design. We then assessed the accuracy and precision of survival estimates from a Cormack-Jolly-Seber model (“CJS”, Pollock et al. 1990) using these input data.

Apparent Survival Rate Estimation using program MARK
In most survival studies, animals are uniquely marked allowing the fates of individuals to be monitored through time. However, we were interested in survival patterns of humpback chub < 100-mmTL which were too small to be marked individually necessitating the use of batch marks (Pine et al. 2013). Batch marks present a peculiar problem in developing capture histories because individuals are not discernible from one another. However, compilation of capture histories is possible because recaptured individuals received new marks and could then be subtracted from the number of individuals available with only the recapture mark. For example, in a three pass capture-recapture experiment, if 200 fish were captured and given a mark on the first trip, then 50 of those were recaptured on the second trip, and 12 of these 50 were then recaptured on the third trip (thus having been caught on all three trips) the simplest capture histories would look like this (capture history, number caught):

- 100 130  130 humpback chub caught on Trip 1 only
- 110 38   38 humpback chub caught on Trips 1 and 2 only
- 111 12   12 humpback chub caught on Trips 1, 2, and 3

where 130 fish were captured only on trip 1, 38 fish on trips 1 and 2, and 12 fish on trips 1, 2, and 3. This could be further extended such that if twenty of the individuals marked on the first trip but not seen on the second trip were recaptured on the third trip, their capture histories would be

- 101 20   20 humpback chub caught on Trips 1 and 3 only

These individuals could then be subtracted from the first group of individuals that were formerly seen only on the first sampling occasion. This logic was extended for all possible capture histories from our sampling trips.

We then developed a suite of *a priori* models to test hypotheses about the probable effects of the annual steady flow experiments based on these capture histories. These models were based on biologically relevant hypotheses about humpback chub behavior coupled with discharge information (Table 1). To convert sample intervals over our 12 day sampling trip to annual apparent rates we calculated sampling intervals as fractions of a year in program MARK (White and Burnham 1999). These intervals spanned from the average date of capture for humpback chub in a given trip to the average date of capture for humpback chub on the next
trip. Annual survival estimates correspond to a “fish year” from July(\(t\)) to July(\(t+1\)) to follow each sampling year. These models were fit to our simulated data and later to our field data based on simulation results.

Model selection

The best models fit to the data were those that were the most parsimonious and biologically reasonable models that also had AIC\(_c\) support (Akaike information criterion for finite sample size; Burnham and Anderson 2002). Models with \(\Delta\text{AIC}_c\) scores less than four units from the top model were considered strongly supported with limited ability to separate inference (Burnham and Anderson 2002). Models with \(\Delta\text{AIC}_c\) between five and ten units from the top model considered moderately supported by the data, and models with \(\Delta\text{AIC}_c\) greater than ten considered poorly supported.

Abundance of juvenile humpback chub from batch marks

We estimated abundance of juvenile humpback chub (40-99 m TL) using sequential Bayesian closed population models described by Gazey and Staley (1986). On each sampling pass of either hoopnets or electrofishing, untagged fish were given marks and released. On subsequent passes, recaptures of marks given that same trip were tallied, and any newly captured individuals were given the same mark. This method resulted in a cumulative increase in the marked population, allowing for abundance estimates to be derived for each pass. In the Gazey and Staley (1986) framework, the distribution of the population size is unknown and assumed to be represented by the non-informative discrete uniform distribution (i.e., prior distribution),

\[
P(N_i) = \frac{1}{K},
\]

where \(P\) is the probability, \(K\) is the estimated discrete number of animals in the population \((N_i=1, 2, \ldots K)\). We assume that \(N_i\geq\) the number of animals marked in the time interval over which closure is assumed (a trip). The number of animals marked each trip, captured each trip, and recaptured are then combined with the prior distribution to create the posterior distribution (i.e., the probability of each possible \(N_i\) given the data) by using Bayes’ theorem (Gazey and Staley 1986). In this framework the posterior distribution becomes more concentrated (i.e., smaller distribution) about the true population size with a normal
distribution as the number of sequential samples increases, or the number of animals captured at each time increases. We calculated the mode of the posterior distribution as the maximum likelihood estimate (MLE) of the sampling distribution to determine the MLE of the population estimate (Gazey and Staley 1986). The 2.5% and 97.5% values (quantiles when normal) are reported as approximate 95% credible intervals. Our basic assumptions for this approach are the same as for other closed population models including (1) marks are not lost or overlooked within a trip, (2) there is no immigration to or emigration from the study reach within a trip, (3) mortality within a trip is assumed to be zero, and (4) recapture probability of marked individuals is assumed to be equivalent to capture probability of unmarked individuals (Pine et al. 2003).

We also derive abundance of juvenile humpback chub using methods described in Williams et al. (2002) where catch is divided by capture probability. We used the same input data as in the survival analyses to delineate our catch. It is important to note that unlike the sequential Bayes’ estimate, the data used for this abundance estimate spans multiple trips and includes all juvenile humpback chub from first capture ≥40 mm (receive a VIE tag) until their body size exceeds 99 mm TL at which point they received a PIT tag and were “removed” from the study population (up to 150+ mm TL). This population will be larger than that considered in the sequential Bayes’ closed capture approaches described above, where all fish are <100 mm TL. Capture probability estimates used were from the most supported Cormack-Jolly-Seber (CJS) model.

Results
Simulation results

Simulations demonstrated which combinations of the CJS model provided relevant parameter estimates based on model convergence and acceptable precision in apparent survival and capture probability estimates. For example, we found the most precise estimates of apparent survival came from models where this was a shared parameter across annual, seasonal, or flow treatments. In all cases, model estimates did not converge where apparent survival was considered independently for each time interval (i.e., for a single flow treatment within a year; Appendix 1).
Field results

Over the three-year duration of this project, we captured and VIE marked 5,575 juvenile humpback chub. Average catch per trip based on calendar year was 221, 331, 588, and 794 for 2009, 2010, 2011, and 2012, respectively. Twenty-seven percent (1,479) were recaptured at least one time. Of the marked cohorts from the first two years of the study, a remarkable 11% were only recaptured after at least one year at large, a surprising result considering the current paradigm that the Colorado River is a mortality sink for juvenile humpback chub.

Estimating abundance of humpback chub < 100 mm TL (sequential Bayes’ framework)

Using closed population models for juvenile humpback chub < 100 mm TL, the MLE of our capture probability estimates ranged from 0.05 - 14% per pass with either gear (Figure 5). Our best closed population maximum likelihood estimates of abundance (based on convergence of confidence intervals around estimates) using electrofishing recaptures ranged from 338 - 1981 humpback chub <100mm TL over the 4 km study reach with 95% credible intervals of 188 - 4,175 (Figure 6; note September 2011 estimate of 5,000 humpback chub did not converge due to low recapture rate). The best abundance estimates based on hoopnets (in the upper 2.5 km area) ranged from 550 - 2,248 humpback chub with 95% credible intervals of 175 - 4,042 (Figure 7, non-convergence in August 2010 due to low recapture rate). In general, abundance in our study reach ranged between 500 and 1,000 individuals until the last fish year (July 2011-July 2012) when abundance doubled to around 2,000 individuals.

Estimating abundance and survival of humpback chub <100 mm TL (CJS framework)

Using open population models (Cormack-Jolly-Seber, CJS) for juvenile humpback chub (≥40 mm TL until first capture ≥100 mm TL; a larger sample population than closed models described above), the maximum likelihood of our capture probability estimates ranged from 6 - 19% per NSE trip with 95% credible intervals from 4 -22%. It is an important distinction that these capture probabilities are for all cumulative passes (both electrofishing and hoopnets combined) on a given trip as well as reflecting the probability of capturing a fish at least once on a trip (no distinction between multiple recaptures), as opposed to a single pass of a single gear with the closed abundance estimates described above. Abundance estimates derived from
catch/capture probability ranged from 1,537 - 7,006 humpback chub over the 4 km study reach with 95% credible intervals from 1,197 - 9,142 individuals (Figure 8).

Apparent survival of humpback chub was not related to flow treatments as the best fitting models were ones that included annual terms for survival but not flow effects. The best supported survival model (based on $\Delta AIC_c$) suggests that apparent survival of juvenile humpback chub declined annually between “fish years” (July-July) 2009 - 2011. This decline was significant with estimates of 69, 46, and 30% in 2009, 2010, and 2011, respectively, with non-overlapping confidence intervals. Additional years of data are necessary to determine whether this represents a declining trend or reflects natural temporal fluctuations in survival rate. Models that included flow terms were either not supported by the data (i.e., $\Delta AIC_c > 10$, Table 1), were not significantly different between flow treatments, or did not improve in model fit after including a flow term. For example, results from a model that had three terms, one for fluctuating flow periods, one for the transition period between fluctuating flows and one for steady flow periods at first appear to show large declines in apparent survival during steady flow periods (Figure 10). However, the model is parameterized such that the same survival rate is estimated across all steady flow periods, and because steady flows occurred during all of 2011 (3 intervals) the lower annual survival of 2011 compared to 2009 - 2010 leads to a lower overall estimate of survival across all steady flow periods regardless of year. We were unable to reach model convergence for a model with both year and flow treatment effects to compare separate steady flow treatments for each year.

Discussion

We did not find significant changes in apparent survival rate of juvenile humpback chub during the steady flow experiments from Glen Canyon Dam during 2009 - 2011. This result differs from the expectation that steady flow periods would improve survival by stabilizing available habitats, potentially reducing energetic costs and predation risks associated with juvenile fish movement. In a companion paper (Finch et al. 2013) we documented a similar counterintuitive response where we found that growth rates of juvenile humpback chub were actually higher during fluctuating flows compared to steady flow periods. While the reasons for this difference in growth between the two flow regimes are unknown, potential factors include
increased availability of invertebrate drift as a prey resource during fluctuating flows compared
to steady flows. In both cases it is important to assess why the direction of change in the
response variable (fish growth or survival) was not as anticipated to help refine our
understanding of juvenile humpback chub ecology and inform future management experiments
and flow policy tests.

Why did survival rates not change in response to the flow experiment? There are
several limitations of the experimental design that may have contributed to this outcome. The
contrast between flow operations in July-August and September-October was relatively small
and coupled with the short time frame may have not been sufficient to produce measureable
effects on humpback chub survival. One of the postulated benefits of stabilizing nearshore
habitats under steady flow operations was increased primary and secondary production,
primarily from backwater habitats (Grand et al. 2006; USFWS BO 2008), but also from cobble or
larger substrates (Blinn et al. 1995). This increased production is thought to benefit fish due to
the potential for food limitation, evidenced by high consumption rates of fish within this section
of river (Cross et al. 2013). The steady flow experiment corresponded with the tail end of
monsoonal flooding in the LCR, a time of increased turbidity and potentially decreased
autochthonous production within the mainstem. Therefore the short duration and timing of
the steady flow experiment may not have allowed increases in primary and secondary
production to be realized.

Previous investigations have demonstrated that in some river systems juvenile or larval
fish, that often occupy shallow water habitats, are most affected by elimination of fluctuating
flows (Freeman et al. 2001; Gaudin 2001). Fluctuating flows affect these small life stages due to
factors such as stranding (Halleraker et al. 2003), downstream displacement (Young et al. 2011),
and dewatering effects on nest survival (McMichael et al. 2005; Grabowski and Isely 2007). It is
possible that the size of juvenile humpback chub studied in this project were large enough to
avoid stranding and dewatering effects, leading to the apparent lack of a response in survival
rates in this flow experiment. Additionally, the lack of a flow related response could be
associated with the persistence of suitable shoreline habitat under both of the flow regimes
observed. Korman et al. (2004) examined the amount of suitable shoreline habitat under
different dam operations and concluded that although fluctuations decreased the amount of persistent shoreline habitat, suitable habitat was available over a large range of flow conditions. The shallow water habitats in which fluctuations are most likely to have large effects were rare in the study reach. Negative impacts resulting from shallow water habitats may be limited in larger regulated rivers.

During 2008-2011 the steady flow experiment occurred during September and October each year. One of the potential benefits of the steady flow experiment was to increase nearshore water temperatures to create a more favorable growth environment for juvenile humpback chub. However, Ross and Grams (2013) did not find warmer water nearshore compared to offshore during the steady flow experiment. The depth and orientation of this reach of the Grand Canyon during these months exclude maximum solar insolation (Yard et al. 2005) which could limit primary production and thermal warming. Additionally, high precipitation during winter 2010-2011 caused large spring inflows into Lake Powell which triggered a flow policy to transfer water from Lake Powell downstream to Lake Mead. These “equalization flows” triggered sustained flows at a higher level during July and August 2011 compared to the fluctuating flows observed in 2009-2010. During September and October 2011 steady flows also occurred (the same months as in 2009 and 2010) but at lower flow levels than observed during July and August (Figure 3). Thus in 2011 there was basically one long time period of 6 months of total steady flows, but at two different flow levels: a high steady flow for four months of the year and a lower, steady flow period for 2 months of the year. This unexpected flow treatment created higher contrast in terms of both flow volume and duration than what was observed during 2009-2010. Yet even with greater contrast in flow volumes and duration of the steady flow treatment in 2011, survival in this year was the lowest of the three years assessed (Figure 9).

Diel variations in flow represent a specific form of modification that alters the physical conditions within rivers (i.e., depth, velocity) creating unnatural and altered habitat conditions (Fette et al. 2007). Juvenile fish that predominately use nearshore or shallow areas are thought to be most affected by fluctuations (Gaudin 2001). One study of the Tallapoosa River below Harris Dam in the southeastern US documented reduced amounts of shallow water habitat and
decreases in young-of-year fish abundance, which resulted in changes in the fish community composition (Freeman et al. 2001). Despite the detrimental effects of diel fluctuations on habitat there is wide variation in ecological responses (Murchie et al. 2008) and individual species responses (Steele and Smokorowski 2000; Young et al. 2011) to the elimination of daily fluctuations. This wide variation in responses to stabilization of flows often necessitates an experimental approach to determine how a particular system may respond. The results presented here highlight the value of empirically assessing species responses to elimination of fluctuating flows.

Why did juvenile humpback chub survival rates change over time in this study? An initial reaction may be that the survival estimates for 2011 are wrong and are confounded with the capture probability estimates. However, our simulation results highlight that the CJS model used to assess survival (the open portion of the robust design model) is likely not biased given the sampling interval, capture probability, and survival rate used in our simulation study and estimated from field data. Ecological factors that could lead to changes in survival over this time period include potential changes in prey resources, density dependent effects, or predation. In March 2008 a high flow experiment was conducted from Glen Canyon Dam that triggered several important ecosystem responses. First, survival rates of juvenile rainbow trout in the Lees Ferry reach below Glen Canyon Dam (approximately 100-km upstream of our study site) increased substantially following the flood in 2008 and their survival rates remained higher than normal even a year later in 2009 before declining to the pre-flood levels in 2010 (Melis et al. 2011; Korman et al. 2011). This improvement in rainbow trout survival is likely due to improved spawning habitat coupled with increased prey availability. Second, shifts in aquatic invertebrates were also observed in response to the 2008 flood event, with large declines in New Zealand mudsnail *Potamopyrgus antipodarum* and amphipod *Gammarus lacustris* production and large increases in drift invertebrate species Chironomidae and Simuliidae. Cross et al. (2013) found that the proportion of invertebrates consumed by fish increased with distance from Glen Canyon dam. Cross et al. (2013) also found high interaction strengths between fish and prey because of low secondary production of prey items combined with very high consumption of available items by fish. The vast majority of humpback chub spawning
occurs in the Little Colorado River about 100-km downstream of the Lees Ferry reach so changes in spawning substrate in the Lees Ferry reach of the Colorado River are unlikely to benefit humpback chub spawning. However it is possible that juvenile humpback chub in the mainstem Colorado River did benefit from increases in the invertebrate community in 2008 and 2009 before these benthic and drift species declined in 2010.

Van Haverbeke et al. (2013) documented long-term trends in native fish including humpback chub ≥150-mm TL and ≥200-mm TL in the Little Colorado River. These authors found trends of increasing humpback chub abundance during spring and fall sampling from about 2006 – 2011, with some of the highest abundance estimates since 2000 occurring in 2009 - 2011. These authors also documented large increases in relative abundance of native flannelmouth sucker *Catostomus latipinnis* and bluehead sucker *Catostomus discobolus* since 2006. These authors suggested that a system-wide change had occurred in this reach of the Colorado and Little Colorado rivers that lead to large changes in population size of these 3 native species. In a project documenting foodweb structure in the Colorado River in 2006 – 2009. Cross et al. (2013) identified the area near the confluence of the Colorado River and Little Colorado River as having the highest number of fish species of the six areas they assessed over 387-km of the Colorado River below Glen Canyon Dam. We found our highest abundance of juvenile humpback chub in 2011 in the mainstem Colorado River and these fish are likely juvenile humpback chub that egressed from the Little Colorado River from the May/June to September/October 2011 Little Colorado River sampling reported by Van Haverbeke et al. (2013). During 2011 the potential for high abundance of juvenile humpback chub (as well as other native fish species) likely existed in a river reach of the mainstem Colorado River where prey consumption rates by fish were previously found to nearly equal or exceed invertebrate production (Cross et al. 2013), and at a time period when prey resources (e.g., invertebrate drift) were declining from the elevated levels following the 2008 experimental flood (Cross et al. 2011). These conditions could lead to density dependent competition for prey resources, preferred habitats, and overall foraging arenas. Chapman (1966) documented that increases in juvenile fish density could lead to displacement of fish into less favorable habitats. Our estimates of apparent survival include emigration (apparent survival = 1-true survival-
emigration) so it is possible that humpback chub in our study reach could have simply emigrated downstream and out of our study reach where we were not permitted to sample. If these fish join the spawning migration to the Little Colorado River in subsequent years as adults they could be identified based on their tag recaptures. Once these fish are identified, the 2011 survival rates could be revised as part of the standard adult humpback chub assessment program (Coggins et al. 2006a).

If these fish did not emigrate from our reach, then another potential mechanism for the low survival in 2011 is direct or indirect effects of predation by rainbow trout. Interactions between rainbow trout, brown trout *Salmo trutta*, and humpback chub in this reach of the Colorado River has been an area of significant research including a multi-year (2003 - 2006) large scale (15-km) removal project for nonnative fish (mostly trout) near the confluence of the Little Colorado River (Coggins et al. 2011; Yard et al. 2011). Yard et al. (2011) found that brown trout had a higher incidence of piscivory than rainbow trout in this river reach where the removal took place. However, since rainbow trout were found in much higher abundance (about 50x), the cumulative predation effect from rainbow trout on humpback chub was much higher. This only measures direct predation rate and does not include any negative effects on humpback chub from predation risk which can have the same consequence as actual predation (He and Kitchell 1990; He et al. 1993). The removal efforts for rainbow trout and other non-native species were highly effective (Coggins et al. 2011); juvenile native fish abundance within the removal reach concurrently increased as non-native fish were removed. However, it is not known whether the positive response in native fish was due singularly to the removal efforts or to a system wide decline in rainbow trout abundance and increases in mainstem water temperature related to drought (Coggins et al. 2011). In our study, catch-rates of rainbow trout increased in each year of our field work; overall they increased by a factor of 4 from 2009 - 2011 (Figure 11). Electrofishing capture probabilities of rainbow trout (J. Korman, Ecometric, Vancouver, BC, personal communication) have been found to remain relatively consistent across trips, trout densities, and turbidities of about 21-27% (average 24%) so it is very likely that our catch rate trends are reflective of actual trout abundance in our study reach. Thus humpback chub survival and rainbow trout abundance are likely negatively correlated, yet our
abundance estimates for 2011 suggest higher abundance for juvenile humpback chub than in 2009 or 2010 when survival was higher and abundance was lower. The interaction between trout and humpback chub is a key area for continued research and experimental management.

Prior to this study, assessments of the status and trends in juvenile humpback chub populations, including population responses to management actions and flow experiments, were primarily based on catch-rate metrics and reconstructed trends in recruitment and abundance based on recaptures of tagged adult humpback chub (ASMR, Coggins et al. 2006a).

We recommend that our direct estimates of abundance and apparent survival be compared to the reconstructed estimates from the standard adult humpback chub assessment program (Coggins et al. 2006a) to see whether trends in survival and abundance are similar between the methods, each with their own assumptions and likely biases. Additionally, our estimates of juvenile humpback chub abundance are derived for specific areas of the mainstem Colorado River (rmk 102.1 to 104.7). In contrast, the reconstructed abundance estimates from the adult assessment estimate all juveniles of the Little Colorado River spawning aggregation. The quotient of these two estimates (NSE abundance estimates/ASMR abundance estimates) would represent the proportion of juveniles that are rearing in the study reach of the mainstem Colorado River and subject to management actions such as experimental flows from Glen Canyon Dam. This information would be important to direct management actions to have the largest potential return to the humpback chub population in Grand Canyon (Limburg et al. in-review).

Acknowledgements

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and thank Humphrey Summit Support and GCMRC logistics for their many hours of hard work
to make this project possible. We thank the University of Florida and the Florida Cooperative
Wildlife Research Unit for administrative and technical support.
Table 1. Apparent survival models for juvenile humpback chub in Grand Canyon from 2009-2012 ranked by ΔAICc score. Model parameter estimation is symbolized as follows: constant (.), time-dependent (t), fish-year (year), flow-dependent (flow), modified flow-dependent (mod_flow), and year-flow interaction (year*flow).

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Models that did not converge

Phi(t) p(all models)
Phi(year*flow) p(all models)
Table 2. Capture-recapture summary table of data provided to MARK for analysis.

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Figure 1. Map of study area near confluence of Colorado and Little Colorado Rivers in Grand Canyon, northern Arizona, USA. Black box denotes the area containing the Little Colorado River aggregation of humpback chub *Gila cypha* and black dots represent the river kilometer (rkm) measured from Lee’s Ferry, Arizona.
Figure 2. Typical contrast observed during steady flow experiments of 2009 and 2010. This graph depicts Colorado River discharge between August 22$^{nd}$ and September 10$^{th}$, 2009 at the Lee’s Ferry gauge, 98 km upstream from this study. The period before September 1$^{st}$ is representative of normal hydropower operations, while the period after September 1$^{st}$ is representative of the steady flow experiment. Data from USGS instantaneous data archive, http://ida.water.usgs.gov.
Figure 3. Daily discharge in the Colorado River at Lee’s Ferry and in the Little Colorado River near the confluence with the Colorado River over the three-year period of this study (2009-2011). Sampling intervals are represented by the diagonally striped boxes. Data from USGS discharge gauges available at waterdata.usgs.gov (Accessed February 2012).
Figure 4. Temperatures of the mainstem Colorado River at Lee’s Ferry and the Little Colorado River near the confluence with the Colorado River. The top panel displays Colorado River mainstem daily water temperatures from prior to the completion of Glen Canyon Dam (vertical grey line) to present, with the time period of this study outlined by the black box. The middle panel displays mean annual temperatures for the Colorado River (CR) over three time intervals, as well as mean annual temperature for the Little Colorado River (LCR). The bottom panel displays daily temperatures of both the COR and LCR for the three year period of this study, as well as the mean annual COR pre-dam temperature as a reference point.
Figure 5. Likelihood estimates of juvenile humpback chub <100-mm TL abundance from hoopnetting data collected for Site 1, Trip 1 (July 2009). Thick black line represents the maximum likelihood estimate (MLE) of abundance after 12 nights of hoopnet sampling while the thin horizontal line represents the (unconverged) likelihood estimate of abundance after 1 night of sampling. Other thin lines represent likelihood estimates after sequential nights of hoopnetting showing that a minimum of 7 nights of hoopnetting were required before a credible (i.e., dome shaped) estimate of abundance were made. With increasing samples (nights of hoopnetting) likelihood estimate becomes better defined and resulting MLE is plotted as the think black line.
Figure 6. Abundance estimates for juvenile humpback chub (<100-mm TL) in the approximately 4 km NSE sampling reach based on electrofishing recaptures during summer and fall sampling trips July 2009-July 2012 using a simple sequential Bayesian closed capture model. Estimates from 2012 included only the upper 2.5 km of the NSE sampling reach.
Figure 7. Abundance estimates for juvenile humpback chub (<100-mm TL) in the NSE sampling reach based on hoopnet captures during summer and fall sampling trips July 2009-July 2012 using a simple sequential Bayesian closed capture model. Estimates during 2009 include only the upper 1.5 km, which was expanded during 2010-2012 to include the upper 2.5 km.
Figure 8. Abundance of VIE-tagged juvenile humpback chub using a Cormack-Jolly-Seber model, summer and fall sampling trips July 2009-July 2012. This is a combined estimate from both hoopnet and electrofishing gear types in the upper 2.5 km of the NSE sampling reach. Individuals were included from when they were first VIE-tagged until they were first PIT-tagged (40 to ≥100 mm TL).
Figure 9. Apparent survival estimates (triangles) and 95% confidence intervals (lines) for juvenile humpback chub from the NSE sampling reach in the mainstem Colorado River from 2009-2012. This is the best fitting model to the data base on AIC$_c$ evaluation and demonstrates that survival rate was similar within a year but different between years. Individuals were included from when they were first VIE-tagged until they were first PIT-tagged (40 to ≥100 mm TL).
Figure 10. Apparent survival estimates for juvenile humpback chub (<200-mm TL) from the NSE sampling reach in the mainstem Colorado River from 2009-2011. This model is parameterized to allow for different flow treatment terms (fluctuating flow, transition flows between fluctuating and steady flows, and steady flows) using the same terms across years. This is important because the largest number of steady flows occurred in 2011, and this is the year of lowest survival according to the most supported model (Figure 8), which reduces the survival estimate for all steady flow periods. We were not able to parameterize a model with unique flow and year affects. Individuals were included from when they were first VIE-tagged until they were first PIT-tagged (40 to ≥100 mm TL).
Figure 11. Rainbow trout total catch per NSE sampling trip (all gears combined) in the NSE study reach of the mainstem Colorado River from 2009-2011.
References


Steele, R. J., and K. E. Smokorowski. Review of literature related to the downstream ecological effects of hydroelectric power generation. Fisheries and Oceans Canada, 2000.


Appendix

Figure A-1. Survival/apparent vs. capture probability simulations using mark/recapture data generated in GENCAPH and analyzed using Program MARK: \( \Phi(t) p(.) \) and \( \Phi(.) p(t) \) models. Note failure to estimate apparent survival for \( \Phi(t) \) models (estimates are either unbounded or missing).

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Figure A-2. Survival/apparent survival vs. capture probability simulations using mark/recapture data generated in GENCAPH1 and analyzed using Program MARK: Phi(t) p(t) models. Note failure to estimate apparent survival for all models (estimates are missing or unbounded).
Figure A-3. Survival/apparent survival vs. capture probability simulations using mark/recapture data generated in GENCAPH1 and analyzed using Program MARK: Phi(flow) p(t) models. Note model accuracy in estimating apparent survival despite order of magnitude change in capture probability due to experimental replicates (years).
Figure A-4. Survival/apparent survival vs. capture probability simulations using mark/recapture data generated in GENCAPH1 and analyzed using Program MARK: Phi(flow) p(flow) models. Apparent survival estimates are accurate if capture probability effects of flow are present, although some precision is lost with capture probabilities <10%. Field data is strongly unsupportive of p(flow) models over p(t) models (Table 3).
Assessing native fish response to whole river warming: Do length-weight relationships change?

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Abstract

Humpback chub *Gila cypha* are an endangered species central to an adaptive management program assessing Colorado River water releases from Glen Canyon Dam into Grand Canyon, Arizona. A portfolio of management actions including non-native fish removal, changes to water intake structures to reduce hypolimnetic water releases from the dam, and reductions in hydropower peaking operations have all been assessed as potential approaches to promote population recovery of humpback chub. In recent years humpback chub populations in this river reach have increased, possibly due to planned and unplanned factors including removals of non-native species and reservoir conditions that have created warmer than normal post-dam water releases. We assessed juvenile humpback chub response to these changes over time through comparisons of humpback chub length-weight relationships and condition in the Colorado and unregulated Little Colorado rivers. Our results demonstrate that the length–weight relationship and condition for juvenile humpback chub in Grand Canyon does not appear to have changed during the recent warm water period compared to the cooler temperatures from the mid to late-1990’s. In the Little Colorado River, however, larger juveniles are heavier today for a given length compared to earlier time periods, despite being uninfluenced by warm dam discharges. The reason for this increase in condition is unknown but may reflect changes in the food base, growing conditions, or lipid accumulation in the Little Colorado River. Condition and survival are thought to be related for other Colorado River native fish and if a similar relationship is shown for humpback chub, then monitoring length-weight relationships could provide a low-cost technique for assessing response of juvenile humpback chub populations to planned and unplanned experiments.
Introduction

Ecologists are often interested in growth dynamics and patterns of plants and animals because these metrics integrate a large range of environmental and ecological factors, including habitat conditions (Quinn and Peterson 1996; Rosenfeld and Boss 2001), food availability (Aresco and Guyer 1999), and predation risk (Werner and Gilliam 1984). Growth in fish is described in many different ways including static descriptions of length or weight and changes in these attributes over time. These growth metrics are widely used to evaluate how fish populations respond to management actions such as habitat restoration or harvest regulations. Growth metrics are also used to characterize spatial variations in species distribution across different biotic conditions, such as changes in prey availability or gradients in environmental conditions (Nicieza and Metcalfe 1997; King et al. 1999).

One species of conservation concern in the southwestern United States is the endangered humpback chub *Gila cypha*, a warmwater fish endemic to the Colorado River basin. The largest extant population of humpback chub persists around the confluence of the seasonally warm Little Colorado River (LCR) and the regulated Colorado River in the Grand Canyon reach of northern Arizona, USA (Kaeding and Zimmerman 1983, Gorman and Stone 1999). This species requires warm water (temperatures >15°C) for spawning (Marsh 1985, Clarkson and Childs 2000). Most adult humpback chub exhibit a spring spawning migration into the Little Colorado River from the Colorado River while offspring return actively or passively to the Colorado River at various life stages (Gorman and Stone 1999; Limburg et al. *in-review*). The timing and factors influencing outmigration of juvenile humpback chub are not well understood as most life stages (juveniles, sub-adults, and adults) can be found in the mainstem Colorado River and the Little Colorado River throughout the year (Valdez and Ryel 1997; Hayden et al. 2012; Limburg et al. *in-review*). Humpback chub populations declined throughout the 1990’s but have shown an increasing trend in recent years (Coggins et al. 2006; Coggins and Walters 2009). Numerous reasons for the decline of humpback chub populations in Grand Canyon have been proposed including: (1) interactions with
non-native fish (Coggins et al. 2011; Yard et al. 2011), (2) loss of habitat due to flow modifications and sediment entrainment behind Glen Canyon Dam (Converse et al. 1998), (3) non-native parasites (Choudhury et al. 2004; Hoffnagle et al. 2006), and (4) changes in riverine temperatures due to hypolimnetic releases of water from Glen Canyon Dam (Clarkson and Childs 2000). Reasons why humpback chub populations have increased in recent years are unclear (Coggins et al. 2011; Van Haverbeke et al. 2013) but management actions have been conducted to test some of the hypotheses above such as non-native fish removal to reduce potential predators or competitors (Coggins et al. 2011) and flow experiments to modify available habitat (Melis et al. 2011).

An adaptive management program for the operation of Glen Canyon Dam is currently in place to monitor, experimentally manipulate, and assess the effects of river regulation on the downstream ecosystem in Grand Canyon while simultaneously meeting power and water obligations to human users (Hamill and Melis 2012). Recent ecosystem experiments designed to benefit humpback chub have been directed primarily at testing the first two hypotheses involving non-native fish interactions and habitat loss (Coggins et al. 2011; Yard et al. 2011; USDOI 2011). Although raising water temperatures could potentially increase spawning habitat and growth of individual humpback chub in the mainstem river (USDOI; 1994; Petersen and Paukert 2005; Coggins and Pine 2010), manipulating temperature presents dam operation and engineering challenges, leading to inadequate testing of the fourth hypothesis above (GCMRC 2007). Ongoing drought conditions in the Colorado River basin leading to low reservoir levels, followed by warming of water releases from Glen Canyon dam since about 2003 and large inflows to Lake Powell during 2011 create an opportunity to study how humpback chub populations respond to warming water conditions. In this paper we use recent river conditions to assess the effects of increased water temperatures on length-weight relationships and condition of juvenile humpback chub in the mainstem Colorado River without the expense of manipulating dam operations. We describe length-weight relationships for juvenile and sub-adult (<200-mm total length,TL)
humpback chub in the Little Colorado River, an unregulated tributary whose temperature and flow were unrelated to Glen Canyon Dam operations for 8 years from 1978-2011. We then describe these same parameters for juvenile and sub-adult humpback chub in the mainstem Colorado River for 6 years between 1991-2011 (Table 1). We examined whether differences in condition exist spatially and temporally between the two river systems and offer hypotheses related to these patterns and in-stream factors such as temperature and density dependence. Finally we suggest that continued monitoring of the length-weight relationship for juvenile humpback chub may provide additional insight into how a key member of the Colorado River ecosystem in this river reach may respond to planned and unplanned changes in river operations or fish community structure. This information provides a line of evidence within a larger collection of studies (Pine et al. in-review) designed to inform management decisions related to water releases, dam operations, and management actions that could be taken to aid recovery of humpback chub and other native fish populations in regulated river systems globally.

**Study Site**

The Grand Canyon reach of the Colorado River is an approximately 400-km section of river bounded by the two largest reservoirs in the United States: Lake Powell and Lake Mead (Andrews 1991). This river reach is considered an area of cultural, biological and geologic significance by tribal, state, federal, and non-governmental interests in the US; it is protected by the Grand Canyon Protection Act of 1992. Due to hypolimnetic discharge from stratified Lake Powell, the Colorado River within Grand Canyon is stenothermic and cool with average water temperatures post-dam ranging from about 8°C to 10°C (1994-2002), warming to about 8°C to 15°C during years of lower reservoir levels and warmer water releases occurring since about 2003 (Figure 1; Voichek and Wright 2007).

The Little Colorado River is the largest tributary of the Colorado River between lakes Powell and Mead. It enters the Colorado River 126 km below Glen Canyon Dam and 100 km (rkm 100) below Lee’s Ferry. Lee’s Ferry is the accepted river kilometer zero...
(rm 0) by convention. Little Colorado River flows are not regulated by mainstem dams but can be affected by agricultural and groundwater users in the upper reaches. The Little Colorado River supports the largest spawning aggregation of humpback chub and a large portion of the juvenile rearing habitat (Gorman and Stone 1999).

Materials and Methods

Humpback chub have been intensively studied in the Little Colorado River and mainstem Colorado River for more than 3 decades (Valdez and Ryel 1996; Coggins et al. 2006; Gorman and Stone 1999). Following standard fisheries guidelines in Anderson and Neumann (1996) and Froese (2006) we restricted our analyses of length-weight relationships to samples collected in the same season (late summer or early fall) to eliminate seasonal variation in growth or condition. Fish weight has not been collected as part of the standard monitoring procedures of the various fish monitoring programs in Grand Canyon. Different sub-sets of data from humpback chub sampling programs from 1979-2011 were compiled depending on which hypotheses we assessed and data availability (Table 1). Humpback chub were collected in the Little Colorado River using seines, hoop nets, minnow traps, and trammel nets, and in the mainstem Colorado River using seines, boat-mounted electrofishing, hoop nets, minnow traps, and trammel nets (see additional details in Douglas and Marsh 1996 and Valdez and Ryel 1997). Sampling occurred in the lower 13.5 km of the Little Colorado River, and in the mainstem Colorado River between rkm 96 and 200, with the majority of sampling taking place directly downstream of the Little Colorado River confluence. During 2011, all fish were collected with hoop nets from the lower 3 km of the Little Colorado River and from rkm 102 to 106 in the Colorado River. The total length (TL) of each fish was measured to the nearest millimeter (mm) and weight in grams (g) was recorded. As part of standard sampling procedures and permit requirements fish were generally processed at shore-based stations under canopies or rock outcroppings for shade and wind protection to reduce fish stress and minimize distractions for visitors to Grand Canyon National Park. In all cases care was taken to measure and weigh fish out of the wind and on a stable surface to maximize accuracy of all measurements. Length-weight data from before
2011 were obtained from the USGS Grand Canyon Monitoring and Research Center database (USGS-GCMRC, Flagstaff, Arizona).

All river discharge and water temperature data for the mainstem Colorado River were obtained from the USGS gauge 0938000 at Lees Ferry. This is the only mainstem river gauge in this river reach with temperature records covering the same time period as our fish samples. This gauge is located 100 rkm upstream of our study site. While water warming does occur as water flows downstream to our study site, we assumed that this warming rate was similar across years and that water temperature was mostly driven by reservoir elevation and hydrology (Wright et al. 2009). We assessed Little Colorado River flow patterns from USGS gauge 0940200 (the gauge station with the most complete records) and temperature data from USGS gauge 09402300, the only gauge with water temperature records in the Little Colorado River beginning in 1992.

Humpback chub <200 mm TL can represent a wide range of ages depending on collection location and growth rate (Pine et al. in-review). Since growth and condition of humpback chub are likely an integration of the current environment and the resources available over the life of the fish we examined trends in water temperature and flow from 1988-2011 in both the Little Colorado River and mainstem Colorado River to examine environmental conditions likely experienced by these fish across their entire life rather than just the year of capture.

We examined the length-weight relationship for juvenile humpback chub (< 200-mm TL and generally < age-3) using several different approaches. We used the standard length-weight model, \( W = aL^b \) (Equation 1) where \( W \) is fish weight in grams, \( L \) is total length in mm, and \( a \) and \( b \) are model parameters. This model was fit to data for each scenario of interest (described below); \( a \) and \( b \) parameters were estimated using a non-linear optimization routine in program R (R Development Core Team 2012). The standard errors were approximated as the square root of the diagonal elements of the covariance matrix. Approximate 95% confidence intervals were then calculated by multiplying the approximate standard errors by 2 and adding or subtracting these values to the parameter estimate. Model parameters were used to fit curves that describe the
relationship between length and weight across the range of sizes for each month, year, or year-and-river combination assessed.

We followed standard fisheries methodology (Pope and Kruse 2007) using a general linear model approach to conduct an analysis of covariance (ANCOVA) to test for differences in the intercepts and slopes of the length-weight relationship by month, year, or river depending on the hypothesis we were assessing (see below). We applied natural-log transformation of the length and weight data prior to modelling to linearize the relationship between these parameters. Residual plots were examined for each of the linear models to ensure that the assumptions of the ANCOVA were met. Model selection was performed using Akaike information criterion (AIC); the most parsimonious model for each scenario was chosen for interpretation. Parameter values for the best model (lowest AIC) are reported.

We used the parameter estimates for equation 1 from the Little Colorado River (data from a common sampling month of August pooled across years) to calculate a standard weight describing the average juvenile humpback chub length-weight relationship from a river with a natural flow and temperature regime. A relative weight index as a measure of condition was then calculated for juvenile humpback chub \((\frac{W_{obs}}{W_s} \times 100)\) where \(W_{obs}\) was the observed weight for each observation from each river and year and \(W_s\) was the standard weight. Box plots of these relative weights were then plotted to show temporal and spatial trends across years and between rivers. We did not use the published standard weight equation for humpback chub (Didenko et al. 2004) for several reasons. First, although the Didenko et al. (2004) equation is well informed, being based on a large sample size of fish from 9 different humpback chub populations, we are not interested in comparing humpback chub in the Little Colorado River aggregation to a “standard size” humpback chub. Our interest instead is in the relative weight of humpback chub in this one population as a response to a whole river warming situation, not in how they compare to an average size estimated from multiple populations. Second, the datasets referenced by Didenko et al. (2004) differ from our data in several ways. The standard equation in Didenko et al. (2004) was developed
primarily from fish > 200 mm TL whereas our emphasis in this study is on smaller fish (<200 mm). In this study we specifically targeted juvenile fish in both the mainstem Colorado and Little Colorado rivers, whereas the Didenko et al. (2004) study had very few juvenile fish from the mainstem Colorado River. Finally none of the fish from Didenko et al. (2004) were collected during the warmwater period we are assessing.

**Scenario 1: Monthly Changes in the Mainstem Colorado River.**

Length-weight data collected from each month (August, September, and October) of 2011 was used to examine seasonal changes in the mainstem Colorado River (Table 1). Equation 1 was fit to each month and results were used to graphically assess changes in the length-weight relationship. Three linear models, each representing different hypotheses about the length-weight relationship during these months, were fit to the transformed data (Table 2). The first model tested whether there was a relationship between log(wt) and log(TL). The second model included a month term to test whether intercepts differed by month [log(wt)~log(TL)+Month]. The third model included an interaction term to determine whether the slopes differed by month [log(wt)~log(TL)*Month].

**Scenario 2: Mainstem Colorado River Comparisons.**

Equation 1 was fit to six years of data collected during August, September or October and results were used to graphically assess how condition changed between years and sizes of fish (Table 1). The same three ANCOVA models as in Scenario 1 were also fit to the data after log transformation but a year effect was used instead of using a month effect (Table 2).

**Scenario 3: Little Colorado River Comparisons.**

Scenario 2 was repeated for juvenile humpback chub collected from the Little Colorado River. Eight years of data were available from samples collected in a common sampling month, August (Tables 1 and 2).

**Scenario 4: Temporal and Spatial Comparisons Between River Systems.**

Data on juvenile humpback chub lengths and weights collected during the month of August were used to make comparisons between the two river systems during a
common month. Data from August was used because this month was sampled across the largest number of years in both rivers. Equation 1 was fit to the data and these results were used to visually assess the model fit (Table 1). ANCOVA models were fit to these data after log-transformation to test whether the length-weight relationship of juvenile humpback chub differed temporally or spatially between rivers. Models included terms that specified different slopes and intercepts for each river and year (Table 2).

**Results**


**Water temperature and flow variation**

We examined river discharge and water temperatures during 1988-2011 to match the years juvenile humpback chub were likely alive during our study period. The fish used in these analyses were generally born from 1988–1994 or a second time period, from 2009-2011. We compared river discharge and temperature patterns in the mainstem Colorado River and Little Colorado River during these two epochs. In the mainstem Colorado River, we found that the time period from 1988-1994 had significantly lower (Welch two sample t-test, p<0.001) and more variable river discharge than the 2009-2011 time period (Figure 2). During 1988-1994 the mainstem Colorado River temperatures were also much colder and the temperature less variable than 2009-2011 (Figure 1). We found that the time when most of our samples were collected in the Little Colorado River (1991-1994) was a period of significantly higher (Welch two
sample t-test, p<0.001) and more variable annual flow volumes than conditions in more recent years (2009-2011; Figure 3). Water temperatures in the Little Colorado River across this same time period are more difficult to assess because of limited water temperature data from 1988-1994 (Figure 4) but comparisons of water temperatures suggest that water temperature was slightly warmer in more recent years (2009-2011; Figure 4) than 1988-1994, but sparse data make the temperature comparison more uncertain.

Scenario 1: Monthly changes in the Mainstem Colorado River.

In 2011 length and weight data were collected for 406, 410, and 266 humpback chub in the mainstem Colorado River during August, September, and October respectively (Table 1, Figure 5). Model comparison using AIC suggested that significant differences (based on large delta AIC values) in the length-weight relationship existed between months (Table 2, scenario 1). The top ranked model (lowest AIC) included a common slope and different intercepts for each month (Table 3). The fitted curves show that juvenile humpback chub greater than 140 mm TL were heavier in late summer (August) than in fall (September–October) (Figure 5). These results suggest that humpback chub incrementally gain weight at the same rate with respect to length from August through October.

Scenario 2: Mainstem Colorado River Comparisons.

The model most highly supported by the data (lowest AIC) estimated individual slope and intercept terms for each of six years (Table 2, Scenario 2). These data suggest that juvenile humpback chub gained weight with respect to length at different rates during the early 1990’s compared to 2011 with 1991 having the highest slope (Table 3). When curves fit to the mainstem Colorado River data are visually examined, juvenile humpback chub across all sizes weigh less for a given length in 1993 and 2011 when compared to other years (1991, 1992, 1998, and 1999). The largest difference observed was for juveniles greater than about 160 mm TL (Figure 6). Additionally, in 1992 fish <100 mm TL were observed to be heavier for a given length compared with other years.
Scenario 3: Little Colorado River Comparisons.

The model which best fit the data estimated individual slope and intercept terms for all five years (Table 2). Significant differences for the intercept terms were found for all years and there were significant slope terms for 1991, 1994, and 2011 (Table 3) suggesting that the rate of change in weight with respect to length was different in these years than other years. The 2011 data highlights an interesting pattern where small fish (80-100 mm TL) generally weighed less for a given length than they did in previous years (1991, 1992, 1993, and 1994; Figure 6 insert). However for 2011 the slope of the relationship between length and weight changes at about 105 mm TL as evidenced by the inflection point in the curve (Figure 6). Beyond this point, humpback chub become heavier for a given length, particularly fish greater than 160 mm TL when compared to other years (Figure 6).

Scenario 4: Temporal and Spatial Comparisons between River Systems.

The ANCOVA model most supported by the data estimated a unique slope and intercept term for each river and year (Table 2). The top ranked model indicated significant differences for some years in the slope and intercept terms for the length-weight relationship in the mainstem Colorado River and the Little Colorado River (Table 3, scenario 4). Significant differences for the intercept terms were also found for several years (Table 3). This suggests that during these years the change in weight with respect to length is different in the two rivers. Graphical assessment of the length-weight relationship by year and river suggests that the weight of a juvenile humpback chub for a given length appears to be relatively constant in the mainstem but does appear to show an increasing trend in the Little Colorado River (Figure 7). During three years (1992, 1993, and 2011) the relative weights of humpback chub were generally higher in the mainstem Colorado compared with the Little Colorado River (Figure 8).

Discussion

We did not find a significant response in the length-weight relationship or condition for juvenile humpback chub in the mainstem Colorado River during the
warmer water of 2009-2011 compared to earlier years. We did find that in the Little Colorado River juvenile humpback chub appear to be increasing in mass for a given length since at least the 1990’s, despite being uninfluenced by warm dam discharges or other management actions in the mainstem Colorado River. These results suggest that changes in mainstem temperature at the levels observed may not elicit a response in juvenile humpback chub condition. Results also suggest that some change may be occurring in the length-weight relationship in the Little Colorado River for unknown reasons.

Our observed changes in condition within both the mainstem Colorado River and the Little Colorado River may be in response to abiotic or biotic changes within these river systems or an artifact of physiological plasticity within the species. Tracking changes in condition with other observed changes in the ecosystem, such as gross primary production or food availability, would prove beneficial to the understanding of the Colorado River ecosystem as changes to condition often reflect variation in available food resources (Blackwell et al. 2000; Paukert and Rogers 2004). A previous study of adult humpback chub found higher condition in Grand Canyon compared with other humpback chub populations in the upper Colorado River basin (upstream of Glen Canyon Dam; Meretsky et al. 2000) and attributed this result to dam-mediated changes in production and a seasonally stable food base (see Stevens et al. 1997 for food base discussion).

There are two particularly interesting observations in our results. During 2009-2011 water temperatures in this reach of the mainstem Colorado were some of the highest observed in a post-Glen Canyon Dam environment. Elevated water temperatures could lead to increased juvenile humpback chub growth potential (Robinson and Childs 2001; Finch 2012). However, we found that juvenile humpback chub condition did not differ during this warm water period from 1988-1990 when higher reservoir operations led to colder water temperatures. A second interesting observation is that between 1988-2011 juvenile humpback chub condition in the Little Colorado River (generally considered to have a natural flow regime) showed increasing
condition while juvenile humpback chub condition in the mainstem Colorado remained generally the stable. What are some possible reasons for these responses?

Van Haverbeke et al. (2013) found trends of increasing abundance of humpback chub, flannelmouth sucker *Catostomus latipinnis*, and bluehead sucker *Catostomus discobolus* in the Little Colorado River since 2000 with some of the highest abundance estimates occurring in 2009-2011. These authors suggest that a systemwide change occurred in this reach of the Colorado and Little Colorado rivers that lead to large changes in population size of these three native species. Based on field research from 2006-2009 Cross et al. (2013) identify the area of the mainstem Colorado River near the confluence with the Little Colorado River as having the highest number of fish species of their six study sites across nearly 400 km of Colorado River between lakes Powell and Mead. In a companion paper Finch et al. (*in-review*) estimated trends in humpback chub population in 2009-2011 near the Little Colorado River confluence and found the highest abundance of juvenile humpback chub in 2011 for this three-year period.

Combined, these results suggest that the potential existed in the mainstem Colorado and Little Colorado rivers during 2009-2011 for high abundance of juvenile fish (humpback chub as well as other native fish species) in areas where prey consumption rates by fish nearly equalled or exceeded invertebrate production (Cross et al. 2013). Increases in juvenile fish density can lead to increased competition for food or habitat, ultimately displacing individuals into less favourable habitats (Chapman 1966). This competition could affect the length-weight relationship by leading to declining condition (through reduced lipid reserves). Yet our results from the Little Colorado River suggest increases in condition during this period of population increase and stable population levels in the mainstem Colorado River, not declines in condition as would be predicted from density-dependent effects. This suggests that either density-dependent competition is not occurring, or if it is, it may be triggering more juvenile fish in the Little Colorado River to emigrate (the “refugees” in Chapman 1966) to the mainstem in search of lower-density habitats. It is interesting to note that the smallest juveniles in this study (about 80-120-mm TL) have very similar length-weight relationships between the
mainstem Colorado River and the Little Colorado River while divergence in condition appears to occur in fish between about 120-200 mm TL (Figures 6 and 7) suggesting an ontogenetic shift in habitat, prey resources, growth, or increase in competition at this size range.

Since 2003, mainstem Colorado River temperatures have increased from an annual range of about 8-10°C to about 8-14°C because of lower reservoir levels in Lake Powell (Voichick and Wright 2007; Figure 1). For other species of Gila such as bonytail Gila elegans, temperatures of less than 14°C depressed the growth of juveniles in laboratory conditions (Kappenman et al. 2012). In laboratory experiments with larvae and small juvenile humpback chub (<50 mm TL) Clarkson and Childs (2000) found that water temperatures of 10°C led to very little growth in length but at 14°C growth increased substantially. From 1998-1994 mainstem Colorado River temperatures exceeded 10°C only about 5-10% of the time but from 2009-2011 this temperature threshold was exceeded about 55-60% of the days (Figure 1). The 14°C temperature threshold was never exceeded during 1988-1994 in the mainstem Colorado River but was exceeded about 5-10% of the time in the 2009-2011 time period (Figure 1). The potential for increased growth in length as water temperatures reach the 14°C threshold is an area in need of further research.

Juvenile fish growth physiology associated with mainstem temperature changes may help to explain why increased condition was not observed in the mainstem Colorado during 2009-2011. Previous research has demonstrated that fish found in seasonal environments must accumulate sufficient energy to survive periods of low food resources, most often in winter. These energy resources are usually in the form of fat and lipid storage in somatic tissue (Schultz and Conover 1997, Ludsin and DeVries 1997, Biro et al. 2004). Fish at both lower and higher latitudes demonstrate this metabolic trade-off by allocating energy toward a necessary basal body mass to survive the winter under reduced rations (Garvey and Marschall 2003). Conversely, if rations are high, fish in lower, warmer latitudes are found to allocate more energy toward skeletal growth because consumption often exceeds metabolic needs in warmer temperatures. Under
colder water temperatures fish may exhibit a deeper body form for a given length compared with a more attenuated body shape in warmer water. Laboratory research on juvenile humpback chub (Clarkson and Childs 2000) suggests limited growth in length in water temperatures of 10°C. Condition may be higher for juvenile humpback chub in the colder mainstem simply because these fish are not growing in length as they accumulate mass. Juvenile humpback chub in the Little Colorado River may be allocating more energy towards skeletal growth due to the seasonally warmer water (Figure 4). Safran (1992), in a review of body shape and length-weight relationships, highlights the wide variation in standard allometric growth parameters ($b$) in many species.

**Management Implications**

Understanding why humpback chub and other native fish populations have exhibited positive trends in population size in recent years (Coggins and Walters 2009; Van Haverbeke et al. 2013) is a key area of interest to the Glen Canyon Dam Adaptive Management Program to inform dam operations and other management actions to promote native species conservation and recovery. A positive relationship between growth (fish size) and survival has been documented for both wild and cultured fish populations (Lorenzen 1996, 2000) and the relationship is often strongest for juveniles (Walters and Juanes 1993). Similar relationships between condition and survival are less certain (Cone 1989; Springer and Murphy 1990). Relationships between survival and lipid accumulation have been shown in juvenile largemouth bass *Micropterus salmoides* (Ludsin and DeVries 1997) and relationships between condition and lipid reserves have been found in Atlantic cod *Gadus morhua* (Lambert and Dutil 1997) but this relationship in humpback chub has not been established. However, it has been established for another Colorado River native species, Colorado pikeminnow *Ptychocheilus lucius* that lipid content and condition for juveniles both declined during winter growth conditions and both were thought to be important to overwinter survival (Thompson et al. 1991).

It is possible that our finding of increasing robustness in juvenile humpback chub in the Little Colorado River is a function of higher lipid accumulation, which could lead to
higher survival either over winter or when these fish emigrate to the colder mainstem Colorado River. While movement between the Little Colorado River and mainstem Colorado River is well documented for adult humpback chub, it has been thought that larvae and small juveniles emigrating from the Little Colorado River have very low survival in the cold mainstem conditions (Clarkson and Childs 2000; Paukert et al. 2006 but see Hayden et al. 2012, Limburg et al. in-review, and Finch et al. in-review for an alternate view). Recent complementary work on survival of juvenile humpback chub in the mainstem Colorado River (Finch et al. in-review) challenges long-standing observations of limited successful mainstem rearing (Valdez and Ryel 1997, Robinson and Childs 2001) by demonstrating persistence of marked cohorts of fish from 2009-2011. Improvements in survival either in the mainstem Colorado River, Little Colorado River or both could be a key contributor to the recent increases in abundance estimates for the Grand Canyon population of humpback chub (Coggins and Walters 2009; Van Haverbeke et al. 2013). Potentially, lipid content could influence survival of juvenile humpback chub during periods of stress or low food availability. Assessing whether or not lipid levels are related to survival and condition in humpback chub as they are in pikeminnow (Thompson et al. 1991) could create a framework for a relatively low-cost additional line of inference on juvenile humpback chub population response to planned or unplanned experiments through monitoring of juvenile length and weight information.

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Table 1. Summary of length-weight analysis parameters for humpback chub in the mainstem Colorado River (MS) and Little Colorado River (LCR) during August (A), September (S) and October (O) 1991-2011. S1, S2, S3, and S4 are the scenarios discussed in the methods section, while an X designates the use of a dataset in that scenario. The total fish sampled (N) and estimates of the \( a \) and \( b \) parameters including the approximate 95% confidence intervals are given for each scenario.

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<th>Year</th>
<th>Months</th>
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<th>( b ) (Approx 95% CI)</th>
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<td>2.25E-06 2.77E-06 1.73E-06</td>
<td>3.28 3.33 3.23</td>
<td>98</td>
<td>X</td>
</tr>
<tr>
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<td>X</td>
<td>MS</td>
<td>6.55E-07 7.96E-07 5.14E-07</td>
<td>3.53 3.57 3.49</td>
<td>113</td>
<td>X</td>
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<td>2011</td>
<td>X</td>
<td>MS</td>
<td>2.89E-06 3.21E-06 2.56E-06</td>
<td>3.21 3.24 3.19</td>
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<td>X X</td>
</tr>
<tr>
<td>2011</td>
<td>X</td>
<td>MS</td>
<td>4.84E-06 5.42E-06 4.26E-06</td>
<td>3.10 3.13 3.08</td>
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</tr>
<tr>
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<td>MS</td>
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<td>3.01 3.04 2.98</td>
<td>266</td>
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Table 2. AIC comparison table for ANCOVA models fit to each of the four scenarios describing the length-weight relationship of humpback chub in the mainstem Colorado River and the Little Colorado River. RY is a factor that codes for each river and year combination.

<table>
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<th>Scenario</th>
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<th>K</th>
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<td>1</td>
<td>log_length * Month</td>
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<td>42.1</td>
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<td>log_length * Year</td>
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<td>2</td>
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<td>log_length</td>
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Table 3. Coefficient estimates (log-scale) for the top AIC ranked ANCOVA models for each scenario in mainstem Colorado (MS) and Little Colorado River (LCR). Parameter estimates are shown in bold if they were significant at an alpha of 0.05.

<table>
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<tr>
<th>Scenario</th>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Scenario</th>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
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Figure 1. Summary plots of Colorado River temperature (degrees C) measured at Lee’s Ferry, Arizona (USGS Gauge 09380000) and other sources across period of record.

Figure 2. Summary plots of Colorado River discharge (flow, cubic feet/second) measured at Lee’s Ferry, Arizona (USGS Gauge 09380000).

Figure 3. Summary plots of Little Colorado River discharge (flow, cubic feet/second) measured near the mouth of the Little Colorado River (USGS Gauge 09402300).

Figure 4. Summary plots of Little Colorado River temperature (degrees C) measured near the mouth of the Little Colorado River (USGS Gauge 09402300).

Figure 5. Monthly predicted weights of humpback chub in the mainstem Colorado River during August, September and October of 2011. Insert shows enlarged view the length-weight relationship for smaller fish.

Figure 6. Annual predicted weights of humpback chub in the mainstem Colorado River (panel A) and Little Colorado River (panel B) during 1991—2011 including data from August, September, and October. Insert shows enlarged view the length-weight relationship for smaller fish.

Figure 7. Predicted weights of humpback chub during August only for the mainstem Colorado (MS) and Little Colorado River (LCR). The 2011 curves for both rivers are shown in black to highlight this year in comparison to data collected during the 1990s (shown in gray).

Figure 8. Boxplots illustrating the distribution of relative weights ($W_r$) of humpback chub 80 – 200 mm TL for the mainstem Colorado River (MS) and Little Colorado River (LCR) during August of 1991—2011. The relative weights shown correspond to scenario 4.
bold horizontal bar represents the median value for each river and year. The box extends from the 25\textsuperscript{th} to 75\textsuperscript{th} percentiles, and the whiskers extend to the 10\textsuperscript{th} and 90\textsuperscript{th} percentiles.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.
Figure 8.
Differential growth of humpback chub in regulated and unregulated portions of the Colorado River basin


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Abstract
Riverine ecosystems have been altered in many large river systems through dam
development to provide water, power, and navigational benefits to human users.
Conservation actions in these river ecosystems historically focused on minimum
releases of water to downstream ecosystems, but increasingly these approaches are
replaced with “environmental” flows designed to benefit ecosystems by mimicking
natural flow conditions (Poff et al. 1997). While intuitive in their design, the testing of
how riverine ecosystems respond to these flow policies is limited. As part of the ongoing
adaptive management program at Glen Canyon Dam, Arizona, an experimental steady-
flow water release was followed during 2008-2012 to assess whether juvenile
humpback chub *Gila cypha* growth, and ultimately survival, would improve under a more
“natural” flow regime compared to typical fluctuating hydropower flows. Our results are
counterintuitive and suggest that steady flows reduced growth rates of juvenile
humpback chub between 100 and 200 mm total length both when steady flows occurred
within the same year (0.12 and 0.15 mm/day fluctuating to 0.07 mm/day steady) and
within the same season across years (0.12 and 0.15 mm/day fluctuating to 0.07 and
0.09 mm/day steady). Otolith incremental analysis did not reveal a change in growth
rates during the flow experiment, but did demonstrate a change in growth rates when
juvenile humpback chub transitioning from the Little Colorado to the Colorado River. Our
results suggest that optimal management policies for listed species in regulated rivers
may not always be predicated simply on more natural flows.
The study of factors influencing the recruitment of organisms to adult populations has yielded important insight into behavior, life history strategies, and species interactions including competition and predator-prey dynamics. The trade-offs between time spent foraging and avoiding predation has been widely studied and the consequences of different time allocation strategies have been assessed across many creatures including mammals (Carey and Moore 1986, Cowlishaw 1997), birds (Schneider 1984), and fish (Strobbe et al. 2011). To maximize growth as juveniles, individuals generally navigate a high-risk gauntlet between obtaining food resources and seeking refuge, until mortality risk declines with age and reproduction occurs with adulthood (Houde 1987; Chambers and Trippel 1997; Ludsin and Devries 1997; Limburg 2001). For species with indeterminate growth such as fish, optimizing the route through this gauntlet can take multiple paths and ultimately influences all aspects of fish ecology.

Several conceptual frameworks are used to describe the choices and consequences fish encounter as juveniles to minimize mortality risk and maximize growth (Werner et al. 1983; Werner and Gilliam 1984; Walters and Juanes 1993; Ahrens et al. 2012). A key idea in these models is the explicit recognition of the relationship between growth and survival, with fitness improving as a function of increasing body size (Werner et al. 1983, Lorenzen 2006). To survive and reproduce, juvenile fish must make critically important choices between predation invulnerable states (e.g. refugia) where prey resources may be low, and prey and predator rich “foraging arenas” (Walters and Juanes 1993; Ahrens et al. 2012). Numerous examples exist of predation-vulnerable juvenile fish restricting their distributions to relatively small proportions of available habitat where both refugia from predation and adequate food resources are available (Werner et al. 1983; Fraser and Gilliam 1987; Lima and Dill 1990; Limburg 2001; Jensen et al. 2011), even though growth may increase if these spatial restrictions are removed (Fraser and Gilliam 1992; Skalski and Gilliam 2002; Skalski et al. 2005).

Field and model-based testing of these ideas are common (Fraser et al. 1995; Walters and Korman 1999, Gilliam and Fraser 2001; Skalski and Gilliam 2003; Walters and Martell 2004; Ahrens et al. 2012). Regardless of risk, juvenile fish must obtain sufficient prey resources for growth and maturation. Individuals that forage successfully and
simultaneously avoid predation are thought to spend less time at small, vulnerable life stages and are thus able to successfully recruit to the adult population and reproduce. For many species, particularly those with specific management goals to maximize individual or population growth such as recovery plans for endangered species or exploited populations, maximizing growth and minimizing mortality is a common goal.

Many fish species of conservation interest, including well-known anadromous species such as salmon, sturgeon, and shad, (families Salmonidae, Acipenseridae, Clupeidae, respectively) as well as freshwater game fish such as bull, rainbow, and cutthroat trout (Salmonidae) and migratory, non-game listed species such as Colorado Pikeminnow *Ptychocheilus lucius* and Cui-ui suckers *Chasmistes cujus* demonstrate tradeoffs in their life-history strategies by spawning in lower risk (and usually lower prey resource) tributary systems before migrating to more productive mainstem river, lake, or marine habitats as juveniles. Similarly, predation-vulnerable small bluegill *Lepomis macrochirus* frequently select habitat that reduces their growth rate but allows them to reduce predation risk by largemouth bass *Micropterus salmoides* (Werner et al. 1983).

The variety of size-dependent species-level behaviors demonstrated in fish highlights the interactions between growth, survival, and recruitment in juvenile fish. Resource managers have long recognized these types of interactions and manipulated them to achieve a desired management outcome such as adjusting water levels or vegetation density to alter predation and recruitment rates of predators and prey in largemouth bass-bluegill farm ponds (Swingle and Swingle 1967) and reservoirs (Woodward and Noble 1999), although sometimes with counterintuitive outcomes (Pine et al. 2009).

The humpback chub, a cyprinid with a complex life history and endemic to the Colorado River basin, is currently listed as Endangered under the U.S. Endangered Species Act. The largest extant population of humpback chub is found in Grand Canyon, Arizona, in the Colorado River near the confluence of the Little Colorado River (Figure 1). Adult humpback chub migrate from the Colorado River into the Little Colorado River to spawn in the spring, where prey resources for juveniles are lower (Stevens et al. 1997, Oberlin et al. 1999) but non-native predator densities are also lower (Marsh and Douglas 1997, Yard et al. 2011). Adult humpback chub population ecology and demography has been studied in this reach since the late 1980’s
(especially in the Little Colorado River), with the majority of data coming from intensive
tagging during the spawning migration (Kaeding and Zimmerman 1983, Valdez and
Ryel 1997, Coggins et al 2006a); for additional references see Goulet and LaGory
2009), with a secondary fall sampling period. These monitoring programs provide direct
estimates of adult survival and abundance, and reconstructed estimates of juvenile
survival (Coggins et al. 2006a).

Abundance estimates derived from these data show population declines in
humpback chub throughout the 1990’s and early 2000’s, leading to concern over status
of the Grand Canyon population of humpback chub (Valdez and Ryel 1997, Coggins et.
al 2006a). This reach of river is highly regulated by the operation of Glen Canyon Dam,
and modeling efforts (Walters et al. 2000) suggested that Colorado River physical
conditions such as fluctuating hydropower flows, cold summer water temperatures, and
negative interactions with non-native fish such as rainbow trout could reduce vital rates
of juvenile humpback chub. However, which of these hypotheses was most influential
during the population decline could not be arbitrated with available data. In an attempt
to resolve this model uncertainty and directly link management actions with juvenile
humpback chub vital rates, a series of experiments was designed and conducted as
part of the Glen Canyon Dam Adaptive Management Program (Melis et al. 2005).
Planned experiments included removal of non-native trout (Coggins et al. 2011; Yard et
al. 2011), high river flows to increase habitat complexity (US DOI 2008, Melis et al.
2011), and steady flow experiments in 2000 (Ralston 2011) and 2008-2012 (this paper).

A persistent uncertainty in assessing how fish populations respond to changes in
river flow management is whether discharges from dams affect growth of juveniles. If
specific flow operations in Grand Canyon improve growth, then these flow operations
may be used to accelerate the recovery of humpback chub populations by reducing time
spent at vulnerable juvenile sizes with higher mortality and ultimately lead to
improvements in survival, recruitment, and population status (Coggins and Pine 2010).
For example, extant flow operations from Glen Canyon Dam are designed partially to
follow diel fluctuations in power demand across the southwestern United States, with
electricity production increasing and decreasing daily (proportional to dam discharge
and flow rate in the downstream Colorado River through Grand Canyon) and causing an
artificial “tide” along the river corridor. This discharge regime is known as “modified low fluctuating flow,” and the ecological impacts are unknown for most ecosystem components (Korman et al. 2004), including juvenile humpback chub. In contrast, under steady flow conditions and where low-angle habitat is available (e.g. backwaters and shallow vegetated gravel bars) water is retained and warms on a spatially limited scale (Korman et al. 2006), which improves growth rates for juvenile rainbow trout compared to fluctuating flows (Korman and Campana 2009). Similar growth patterns have not been documented in native fish (most of which reside > 48 km downstream of the reach where trout growth was studied), although increased temperatures could potentially improve metabolic efficiency and growth of warmwater species such as humpback chub (Petersen and Paukert 2005; Coggins and Pine 2010). In the context of population recovery, increased growth of juvenile humpback chub could improve survival by reducing the amount of time juveniles spend in critical life stages with high mortality rates (i.e., population bottlenecks) and increase recruitment.

To determine if growth rates of juvenile humpback chub improve during steady flows, Glen Canyon Dam Adaptive Management Program initiated a series of experimental steady water releases from Glen Canyon Dam for specific time periods (September and October 2008-2012; Figure 2) as a contrast to extant fluctuating flows the remainder of the year. Here we test for growth responses to this flow experiment by comparing growth rates of juvenile humpback chub both within and between years in the mainstem Colorado River when experimental, whole river reach steady flow treatments occurred (2009-2011). We also compare these growth rates to those of fish from the unregulated Little Colorado River to assess whether potential changes in growth can be attributed to the flow experiment. By comparing growth both within the mainstem Colorado River and between the regulated mainstem and an unregulated tributary, we improve the current understanding of juvenile humpback chub recruitment in the Colorado River to inform dam operations and other management actions intended to aid humpback chub population recovery.
Methods

Study site.—The Grand Canyon reach of the Colorado River is the roughly 400-km river section bounded downstream by Lake Mead (Hoover Dam) and upstream by Lake Powell (Glen Canyon Dam), the first and second largest reservoirs in the United States, respectively (Andrews 1991, Figure 1). Average discharge of the Colorado River through Grand Canyon for the past decade (2000-2010) was 351 m³/sec (Phantom Ranch gauge, 171 km below Glen Canyon Dam). This canyon area is among the most recognizable natural features on earth, and the cultural, geologic, and biological significance of its resources are internationally recognized through UNESCO World Heritage Site designation as well as legislative protection through the US National Park System and Grand Canyon Protection Act of 1992.

The Colorado River itself within Grand Canyon is stenothermic and cool due to the storage effect of Lake Powell and hypolimnetic water releases from Glen Canyon Dam penstocks. Mean annual Glen Canyon Dam water release temperatures fluctuate around 2°C (from 8°C to 10°C from 1994-2002), with recent water temperatures (2004-2012) fluctuating annually by as much as 7°C (from 8°C to 15°C in 2011, Figure 3) due to rapidly changing reservoir levels due to drought and water policy. However, while water temperatures of 2011 would be considered very high in a post-dam Grand Canyon ecosystem, the temperature range still only spans about 25% of the pre-dam annual temperature fluctuation (Figure 3). This reduction in seasonal mainstem temperature maximums is believed to be one driver for the reduction (humpback chub) or extirpation (bonytail and roundtail chub, razorback sucker, Colorado River pikeminnow) of populations of warmwater endemic fishes in Grand Canyon (Kaeding and Zimmerman 1983, Valdez and Ryel 1997, Clarkson and Childs 2000).

The lower 14 km of the Little Colorado River and the associated inflow reach of the Colorado River contain the “Little Colorado River aggregation” of humpback chub (~90% of humpback chub in the lower basin, Figure 1). The Little Colorado River is the largest tributary of the Colorado River within Grand Canyon National Park, and drains approximately 44,000 km² of northern Arizona and western New Mexico with a mean annual discharge of 11.54 m³/sec since 2004. The Little Colorado River is essentially
unregulated, although some of the upper basin has been dewatered due to human
settlement. Runoff patterns are characteristically bimodal: extended discharges during
snowmelt-dominated spring floods are followed by shorter, stochastic summer flood
pulses associated with monsoonal precipitation (Figure 4). We selected the Little
Colorado River as a control system for humpback chub growth studies because it is the
only unregulated river in the Grand Canyon reach of the Colorado River with an extant
population of humpback chub. The natural seasonal fluctuations in hydrology and
temperature of the Little Colorado River improve our ability to determine whether
potential changes in growth occur naturally due to seasonality or whether changes may
be occurring in the Colorado River due to flow effects.

Sampling techniques and seasons.—We assessed juvenile humpback chub growth
rates using two different approaches; (1) changes in length over time based on
recaptures of tagged individual humpback chub as part of a mark-recapture program in
the mainstem Colorado and Little Colorado rivers and (2) otolith incremental analyses
from juvenile humpback chub specimens provided via cooperating management
agencies. Samples were collected during July-October 2009-2011. July and August
Colorado River samples during 2009 and 2010 represented typical fluctuating
hydropower flows, while September and October samples from all years represented
the fall steady flow experiments (Figure 4) which began on 1 September (Figure 2) in
each year. Large upper basin inflows and regulatory requirements related to water
levels in lakes Powell and Mead also resulted in unexpected steady flow conditions
during July – August 2011 (Figure 4).

Mark-recapture sampling.—We collected juvenile humpback chub for mark-recapture in
the mainstem Colorado River from river km (rkm) 102 to 106 (Figure 5; distances are
measured from Lee’s Ferry at rkm 0, which is ~25 km below Glen Canyon Dam) near
the confluence of the Little Colorado River, and in the Little Colorado River from the
confluence with the mainstem Colorado River upstream roughly 14 km. We generally
sampled 10 to 12 days each month for 4 months each year in both rivers (12 trips total,
Figure 4). Fish collections in the mainstem Colorado River were made using two gear
types: un-baited mini hoop-nets (50 cm diameter, 100 cm long, single 10 cm throat, made of 6 mm nylon mesh, fished for 12 consecutive days over 24 hour intervals, n = 47-80 per trip, fished systematically in our study reach) and slow-speed boat electrofishing (pulsed DC current, 15-20 amps, 200-300 volts, boat speed 7-10 seconds per meter of shoreline, repeated 24 to 72 hours apart for 3 to 5 total passes per trip).

We sampled the Little Colorado River with the same un-baited mini hoop-nets, but because of permitting and logistical constraints we fished these nets in areas likely to have high juvenile humpback chub abundances in order to maximize marks and recaptures of fish for use in growth analyses. Electrofishing was not feasible in the Little Colorado River due to naturally high conductivity.

Following standard Grand Canyon cooperative fisheries research procedures (Ward 2009, Persons et al. 2012), we measured, tagged and returned humpback chub to the same location where they were captured. All humpback chub > 100 mm total length (TL) received a 134.2 kHz passive integrated transponder (PIT) tag (9 mm long, BIOMARK) with a number identifiable to individual fish. While a range of humpback chub sizes were collected during this study, we report only humpback chub large enough to be individually PIT tagged (≥ 100 mm TL) but less than 200 mm TL at the first capture event (“juveniles”). To calculate growth, marked fish had to be subsequently recaptured on the next trip such that any measured growth occurred during the extant conditions in the preceding ~3-4 weeks. This was done to eliminate the influence of inter-annual, seasonal, or ontogenetic shifts in growth rates that may occur. One exception occurred in the Little Colorado River in 2010, where we included recaptures of juvenile humpback chub from both August and September as part of the summer growth group (initially measured in July) due to heavy flooding and low recaptures in August alone. This is not problematic because no marked flow change occurred in the Little Colorado River in September (as opposed to the steady flows in the mainstem beginning September 1), so these individuals still experienced summer growth conditions.

We calculated the mean daily growth for the groups of recaptured humpback chub for each of the 12 combinations of river, flow treatment (month), and year humpback chub were collected (Table 1) over the interval during which they were at
large. We then resampled these growth estimates 10,000 times using a bootstrap procedure with replacement (R Development Core Team) to determine the distribution of the mean growth rate for that subsample. The key assumptions of this approach to characterize growth distribution was (1) fish remain within the same river system for the duration of time between capture and recapture, and (2) captured individuals represent a random sample from the overall population.

Otolith samples.—We also examined otoliths to potentially improve our growth rate inference since otoliths can demonstrate changes in growth over intervals as small as 24 hours (Korman and Campana 2009). Otoliths can also be used to determine growth rates in humpback chub too small for mark-recapture, as well as provide data from periods during the study when field sampling did not occur. Although we did age all humpback chub specimens available at the time of this study, here we only included daily growth data from fish whose approximate age in days was identifiable from otolith rings (age 0 individuals). The older fish demographic whose age in days was not discernible through otolith increment analysis (often >100 mm TL) were instead available for mark-recapture analysis.

Since any directed sampling of humpback chub for otoliths was not allowed due to their listing under the US Endangered Species Act, samples came haphazardly from incidental mortalities that occurred as part of standard fish monitoring activities by cooperating fisheries research agencies. From this collection, all juvenile humpback chub had their lapillar otoliths removed via dissection. Preparation of otoliths for age analyses was adapted from Limburg (1996) where removed otoliths were cleaned in a dilute (10% V:V) bleach-water solution, air dried, embedded in epoxy, and assigned an identification tag. The embedded otolith was then sectioned with a diamond saw in the longitudinal plane and ground to a thickness of 0.05 - 0.2 mm. Final polishing was completed using a series of increasingly fine polishing papers. Otoliths were then examined under a compound light microscope at 100 - 1000x. Counts of otolith increments were made two to three times along the longest growth axis. Counts of daily otolith increments that fell within three to four increments were averaged. Counts that fell outside of this range were re-examined and averaged. Annual rings (if present) were
recorded. Hatch-date was calculated as the date of collection minus estimated age from daily incremental counts for fish < age 1. Mean daily growth rate (DGR) was calculated as DGR = (TLc-7.1)/age where TLc is the total length at collection and 7.1 is the length at swimup (Hamman 1982).

Results: Growth rate estimates

Growth response from tagging data.—We found that juvenile humpback chub daily growth rates were lower during steady flows than fluctuating flows when they occurred in the same season (0.07 mm/day during steady flows from July to August 2011 versus 0.12 mm/day and 0.15 mm/day during fluctuating flows from July-August 2009 and 2010, respectively; Figure 6). Growth rates were also lower when steady flows followed fluctuating flows within the same year (0.08 mm/day and 0.07 mm/day during steady flows versus 0.15 mm/day and 0.12 mm/day during fluctuating flows in 2009 and 2010, respectively; Figure 6). There is some overlap of the bootstrapped resample of mean daily growth rates, but the distributions were essentially disjunct and demonstrated that on average juvenile humpback chub grew faster when discharge fluctuated than during steady flows.

Flow conditions in the Little Colorado River were not related to dam operations (Figure 4), and as expected there were no trends in growth rates for the Little Colorado River related to flow in the mainstem Colorado River (Figure 6). We did observe a seasonal decline in mean daily growth rate in 2009 and 2011 from rates of roughly 0.12 mm/day from July to August/September down to roughly 0.01 mm/day for the interval between September and October. Of the three years assessed, 2010 growth of juvenile humpback chub in the Little Colorado River was relatively high (0.25 mm/day) and did not decline in September and October as it had in previous years (Figure 6). This may be related to possible reduced density after an extraordinarily active monsoon flood season or other unknown factors.

Growth from otolith samples.—We estimated ages for all humpback chub in days for young fish, or years for fish with one or more dense annuli. Samples came from both the Little Colorado River (15-134 mm TL, n = 57) and mainstem Colorado River (18-197
mm TL, n = 52). In the Little Colorado River, all but one juvenile humpback chub < 200 mm TL were age 0 or age 1 fish, while fish < 200-mm TL from the mainstem could be as old as age 5 (Figure 7). Individuals whose age in days was estimable averaged 32.9 mm TL (SE = 2.81) from the Little Colorado River and 54.2 mm TL (SE = 4.4) from the mainstem Colorado River, and were from 26 to 250 days old. Estimated daily growth rate in the Little Colorado River was 0.49 mm/day; Colorado River growth rates were 0.36 mm/day (Figure 7).

While growth and hatch date information was recoverable for most of the otoliths examined, we did not observe a growth response due to the flow experiment, either because it was absent or because any change was below detectable levels. On the other hand, sharp contrasts in growth increments were typically observed in fish moving between the Little Colorado River and mainstem Colorado River (as identified by otolith chemistry; Hayden et al. 2012). Incremental otolith analyses did not reveal changes in growth in the Little Colorado River during July-October, which corresponds with growth estimates from tagging data. Estimated hatch dates based on our ages and collection dates indicated that about 29% of the juvenile humpback chub hatched in April, 42% in May, and 20% in June. The remainder came from spawning in February, March, July or September.

**Discussion**

We found more rapid growth during modified low fluctuating flow periods compared to steady flows. These results were consistent across multiple years and seasons, and are counterintuitive to the expected result of improved growth under a “more natural” steady flow regime. Minor changes in growth and survival rates during early life stages can have large impacts on the number of successful recruits later in life (Houde 1987) because the length of time an individual spends in early life stages (with higher mortality) dramatically influences the recruitment of that cohort to the next life stage. Navigating the various mortality gauntlets of early life more quickly by growing faster can increase the proportion of foraging habitat available as well as expand available prey resources. Larger size has been shown to have survival advantages for juvenile fish specifically because these larger individuals are able to transition to foraging on higher-quality prey items, accelerating growth and lipid accumulation and
improving survival through multiple seasons (Keast and Eadie 1985; Wicker and Johnson 1987; Ludsin and DeVries 1997). Results of this study suggest that humpback chub would spend more time in vulnerable juvenile life stages if short (~2 months) steady flow experiments of the magnitude we observed were inserted amid a fluctuating flow regime.

These conclusions are based on several key assumptions. First, humpback chub used in the growth analyses had to remain in the same treatment group (river and season) between captures (assumption #1, Table 1). If individuals moved between the mainstem and Little Colorado River between captures, then the observed growth rates would not reflect the observed environment for each flow treatment. Our results from otolith growth and microchemistry (Hayden et al. 2012) analyses suggest that during this time of year and over the short time period fish were at liberty between collections (15 to 70 days), it is likely that this assumption was met. For both growth estimation approaches, we assume that the sample is a random and unbiased representation of juvenile humpback chub growth (assumption #2). If slower or faster growing individuals died or emigrated from our sampling reaches prior to our sampling, then these fish would not be included in our analyses because they would be unavailable for recapture.

Otolith incremental analysis indicated that growth of small juvenile humpback chub was faster than growth rates of larger juvenile humpback chub reconstructed from tagging data, which is not surprising. Growth rates for age 0 juveniles in the Little Colorado River were the highest of all fish examined (0.49 mm/day), although it is important to note that these fish were also the youngest (when growth is expected to be fastest) and experienced the warmest temperatures of all fish sampled. Growth of juvenile fish moving between the Little Colorado and mainstem Colorado rivers displayed obvious “check” marks in their otoliths corresponding to movement from that tributary to cooler mainstem waters, indicating that conditions in the mainstem Colorado River are likely less favorable for growth than the Little Colorado River at this body size (Hayden et al. 2012). This contrast could be significant, as cold water affects growth (Clarkson and Childs 2000), swimming ability (Ward et al. 2002), and predation vulnerability (Ward and Bonar 2003). Otoliths from fish that were residents in the Colorado River at the onset of the fall steady flow experiments did not display similarly
abrupt variation in increment width, indicating that if there were any changes in growth
due to the flow experiment, they were likely gradual and insignificant, despite the
visually compelling adjustment in discharge (Figure 2). This demonstrates that the
transition for migrating individuals from the nursery environment of the Little Colorado
River to the environment of the Colorado River is much more influential than the
transition from fluctuating to steady flows within the Colorado River for juvenile
humpback chub already surviving there.

We found that growth in the Little Colorado River was highly seasonal in tagged
fish, with rapid summer growth followed by winter growth rates that were actually lower
than the mainstem Colorado River. Otolith results also demonstrated higher growth in
the Little Colorado River than in the Colorado River, especially for small fish.
Importantly, fish from the Little Colorado River were much younger than similar-sized
fish from the mainstem. Otolith growth rate estimates from the Little Colorado River
were derived from fish age 0 or age 1, while in the mainstem river growth rates from
similar sized fish would surprisingly be from age 0 individuals to as much as age 5.
Although fish exhibit indeterminate growth, this age effect may play a significant and
unknown role in the reduced growth rates we observed for fish of similar sizes in the
mainstem Colorado River. Those individuals that overwintered in the Little Colorado
River and were later used in otolith analyses were also likely the most successful
competitors for food and habitat, and we would expect their growth rates to remain
higher than emigrants. This would not be reflected in tagging data, as mark-recapture
created short-term “windows” into the growth rates of the fish and only included fish
over 100 mm TL, and otolith data integrates growth rates across the fish’s entire
lifespan.

Water temperature has been shown to strongly influence the growth of age 0 fish
(Mooij et al. 1994; Staggs and Otis 1996; Clarkson and Childs 2000). However, the
temperature of water released at Glen Canyon Dam is not directly controllable due to
the fixed withdrawal location of the penstocks on the dam. Given this engineering
design, temperature of discharged water is heavily influenced by the location of the
thermocline in Lake Powell. As this water then flows downstream from Glen Canyon
Dam under summer conditions, it warms at a maximum rate of about 0.02°C/km (8.5°C
in 400+ km) in July at typical flow volumes, but this can increase under extremely low flow volumes (Wright et al. 2009). Under these circumstances, water temperature is primarily influenced by basin level hydrology, which drives the level of Lake Powell and the position of the thermocline coupled with ambient air temperature downstream.

Since about 2003, persistent drought throughout the Colorado River basin (with the exception of winter 2010-2011) led to exceptionally low water levels in Lake Powell and dam discharges from the metalimnion or higher, resulting in a period of much warmer water releases than the previous 20+ years since Lake Powell filled (Figure 3). During the three-year period of this study, temperature profiles were also warm (higher than the post-dam average). In 2011, high spring runoff into Lake Powell necessitated unusual high volume releases to move water to downstream users that not only flooded a great deal of new habitat but were associated with near-record high discharge temperatures (Figure 3). Based on temperature alone, we would expect growth to be highest during all periods of 2011. However, summer 2011 showed the lowest growth rates observed in the mainstem despite being a warm and steady flow period, perhaps related to availability of invertebrate forage in the drift.

Insufficient data precludes direct inference about food availability in our study area, although there is a relationship between insect drift and flow fluctuation in other regulated rivers. In natural systems, insect drift is timed (often nocturnal) to reduce predation by visual feeders such as fish and maximize downstream dispersal (Waters 1972; Muller 1974). Drift in regulated rivers, however, can artificially increase with changes in flow, as has been observed in the Flathead River of Montana (Perry and Perry 1986), the Hawea River of New Zealand (Irvine and Henriques 1984), and the upper Colorado River (Poff and Ward 1991). These pulses of invertebrate drift are likely present in Grand Canyon based on invertebrate responses to flood events (T. Kennedy, USGS GCMRC, personal communication), and may partially compensate for the paradigm of reduced growth due to lower water temperatures. When artificial discharge fluctuations are omitted as part of a management experiment and insect drift becomes more natural, juvenile humpback chub may have to forage more actively, which not only increases their metabolic expenditure but also increases their exposure to predation.
We suggest that higher, warm, steady discharges likely increased energy expenditure both metabolically and behaviorally, but until newly flooded substrates became colonized, invertebrate density in these higher water volumes was reduced. Cross et al. (2011) documented large declines in some invertebrates following experimental floods in Grand Canyon. This colonization may also have been hampered by lower invertebrate diversity in regulated rivers (Vinson 2001; Munn and Brusven 2006). These likely increases in metabolism and reductions in drift density thus reduced growth rate until primary production and food availability recovered, consistent with the trend of low growth rates that we observed in summer 2011. Inversely, the lower, warm, steady flows of fall 2011 showed the highest growth rates for that season, likely due to concentration of invertebrates from the previously larger wetted area into a smaller volume of water. A similar response was also seen in the regulated Kootenai River, Montana, where the highest rates of insect drift were recorded during a lower steady flow immediately following a period of high steady discharges (Perry and Perry 1986).

Management implications

“Natural flows” from Glen Canyon Dam have long been advocated for restoration efforts in the Grand Canyon reach of the Colorado River. Other flow and water policy management actions include a seasonally-adjusted steady flow routine (to mimic spring floods and summer low flow periods) or building a selective withdrawal device to have warm-water discharge below Glen Canyon Dam. These and other intuitively simple management actions are at least partially motivated by the expectation that a more natural Colorado River, similar to the pre-dam environment, would be beneficial to the recovery and persistence of humpback chub through improvements in growth, survival, or recruitment. However, these proposals are costly to implement in terms of lost power revenue and construction costs and the outcomes of either or both policies are still highly uncertain (Lessard et al. 2005), especially considering the (generally poor) population status of native fish in southwestern rivers that still have “natural” flows (i.e., upper Gila, Verde, and Salt rivers). In this study we have demonstrated that at least one objective of a more “natural” steady flow—improvements in growth rates for juvenile
humpback chub—was not observed, and this has direct implications for future experiments as part of the Glen Canyon Dam Adaptive Management Program.

Humpback chub evolved in a Colorado River ecosystem of extreme flow, turbidity, and temperature fluctuations throughout Grand Canyon for thousands of years. Over the last five decades, however, the Colorado River has been characterized by seasonally reduced flow, low turbidity, and narrow temperature fluctuations following the construction of Glen Canyon dam. To persist in the dynamic environment experienced over evolutionary time scales, humpback chub evolved a variety of physical and behavioral adaptations for extreme environmental conditions. Many of these characteristics are likely exaptations (Gould and Vrba 1982) for the modern Colorado River, and strong selective pressures for tolerance of persistent cool water and fluctuating flows are ongoing. Given the historical and extant variations in flow conditions these fish experience, the relatively small variation in flows tested in the current flow experiment should not have been expected to trigger a strong response in fish growth rates, and our results support this conclusion. For humpback chub in the Little Colorado River aggregation, experimental flow treatments from Glen Canyon Dam 125 km upstream are even more reduced because of the attenuation of modified discharges from the dam over the river course as well as the high-angle shorelines (cliff and talus) and deep eddies common below the confluence of the Little Colorado River, which offer relatively consistent habitat types across a range of discharges.

Both steady flows and warmer water temperatures are expected to increase growth rates of juvenile native fish with the expectation that faster growth would result in higher survival. We found that growth rates were actually lower during steady flow periods compared to the extant fluctuating flows. Our project also took place during a period of some of the warmest water temperatures observed in a post-dam Colorado River (Figure 3). We also observed higher growth rates from tagging data in the mainstem Colorado River than in the Little Colorado River during fall (and presumably through the winter). Strictly from a growth perspective, an optimal life history strategy for juvenile humpback chub may be to spend spring and summer in the Little Colorado River, and emigrate to the mainstem Colorado River when Little Colorado River growth conditions decline in winter. This behavior likely increases predation vulnerability
(because of higher predator densities in the Colorado River), but may be offset by reduced time spent at small body sizes. Recent evidence suggests that some juvenile fish do overwinter in the mainstem Colorado River before returning to the Little Colorado River even in their first 1-2 years of life (Hayden et al. 2012). This life history strategy helps explain the wide variation in observed age for a given size for juvenile humpback chub in Grand Canyon. These migratory behaviors between river locations will be assessed in a separate publication.

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Table 1. Sample sizes of juvenile humpback chub used in mark-recapture growth study.

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<td><strong>Colorado River</strong></td>
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<td>2009</td>
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<td><strong>Little Colorado River</strong></td>
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<td>2011</td>
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Figure 1. Map of study area near confluence of Colorado and Little Colorado Rivers in Grand Canyon, northern Arizona, USA. Black box denotes the area containing the Little Colorado River aggregation of humpback chub.

Figure 2. Colorado River discharge August 22\textsuperscript{nd}-September 10\textsuperscript{th}, 2009 at the Lee’s Ferry gauge, 98 km upstream from this study universe. The period before September 1\textsuperscript{st} is representative of normal hydropower operations, while the period after September 1\textsuperscript{st} is representative of the steady flow experiment. Data from USGS instantaneous data archive. Available: http://ida.water.usgs.gov.

Figure 3. Colorado River water temperatures as measured at Lee’s Ferry, 25 km downstream from Glen Canyon Dam. Top figure is daily water temperatures since 1950. The dotted gray line marks the date Lake Powell began filling (closure of GCD), and the black box is the three years included as part of this study. The middle figure is mean water temperatures for three intervals: before the closure of GCD, after the filling of Lake Powell (“cold” water period), and since the onset of drought in 2004 (“warm” water period). Lower figure is the mean water temperature pre-dam, overlaid three times with the daily water temperatures across the three years of this study.

Figure 4. Daily discharge in the Colorado River at Lee’s Ferry and in the Little Colorado River near the confluence with the Colorado River over the three-year period of this study (2009-2011). Sampling intervals are represented by the diagonally striped boxes. Data from USGS discharge gauges. Available: waterdata.usgs.gov (February 2012).

Figure 5. Detail map of study area. Distances from Lee’s Ferry are denoted in kilometers. All available shoreline (excludes rapids) were electrofished in all trips. Site 1 contained 47 hoops on trip 1 and 60 hoops on trips 2-12, deployed across habitat types according to their availability. Site 2 contained no hoops on trips 1-6 and 20 hoops (upper half) on trips 7-12, also deployed across habitat types according to their availability.
Figure 6: Distributions of the mean daily growth rates for juvenile humpback chub (100-200 mm total length), determined with 10,000-iteration bootstrap resamples with replacement. Colorado River samples represented both fluctuating (top left) and steady (top right) flows, while Little Colorado River samples (bottom row) represented unmanaged flows.

Figure 7. Age estimates derived from otolith incremental analysis for fish of a given length. Ages for individuals older than one are presented in gray. Daily growth rate estimates are only derived from individuals whose ages were estimable in days (black). Squares represent fish resident in the Colorado River, while triangles are Little Colorado River fish. The solid line is the modelled growth rate for age 0 fish in the Little Colorado River, while the solid line represents growth rate in the mainstem Colorado River.
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Figure 1.
Figure 2.

2009 Colorado River Discharge
Lee's Ferry, Arizona

flow experiment began annually on September 1

Date
Aug 22 Aug 26 Aug 30 Sep 3 Sep 7
Discharge (cfs)
0 5000 10000 15000 20000
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.
Does a “less modified” river trigger improvements in native fish growth rate? An assessment from the Colorado River

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Abstract

This paper compares growth rates of humpback chub in the Colorado River, Grand Canyon Arizona during two different time periods (Epochs 1 and 2) that differed in hydropower operations, reservoir elevation and river discharges, native and non-native fish densities, and also, possibly preferred prey resources. Epoch 1 (1980-1993) is more “highly modified” of the two time periods with large variation in river flows, colder water temperatures, higher non-native fish populations and lower abundances of native fish. Epoch 2 (2001-2011) represents a “more natural” (but still modified) period with more stable river flows, warmer water temperatures, lower abundance of non-native fish and higher abundances of native fish. Our findings suggest adult humpback chub size at age are lower in Epoch 2, despite warmer water temperatures, lower predator densities, and higher abundances of congenerics than observed in the 1980’s and 1990’s – factors thought to promote faster growth rates. However, our research also suggests that juvenile humpback chub are larger for a given age today than previously observed during the 1980s and 1990s. This finding is surprising as juveniles reside primarily in the Little Colorado River which is not influenced by management actions in the mainstem Colorado River. These results may indicate differential growth responses to changes in the Colorado and Little Colorado river ecosystems between adult and juvenile humpback chub.

Introduction

Riverine ecosystems response to dams and flow regulations from hydrologic, geologic, and ecological perspectives (Ligon et al. 1995; Stanford and Ward 1991; Nilsson et al. 2005) is an important area of research used to inform choices related to dam removal (Shuman 1995; Poff and Hart 2002) and operation of existing dams for conservation purposes (Richter and Thomas 2007; Olden and Naïmen 2010) or to influence policy decisions and conservation actions related to new dam construction (Finer and Jenkins 2012). The Colorado River ecosystem below Glen Canyon Dam, Arizona was significantly altered following the closure of Glen Canyon Dam in 1963. Key changes in this river reach bounded by lakes Powell and Mead affect physical processes below the dam. Reservoir storage and hydropower demands have reduced the seasonality and magnitude of flows (Topping et al. 2003), caused storage of sand
and fine sediments above the dam in Lake Powell (Schmidt et al. 2004; Wright et al. 2005), and drastically altered river water temperature with colder summer and warmer winter temperatures (Vernieu et al. 2005, Wright et al. 2008). These changes have altered ecological foodweb structure and function (Cross et al. 2011; 2013), including proliferation of non-native fish and invertebrates and decline and/or extirpations of several native fish species (Minckley and Deacon 1991).

To promote recovery and long-term sustainability of natural resources downstream of Glen Canyon Dam while adhering to existing water laws and agency authorities, a preferred alternative for dam operations was selected in 1996 (Record of Decision, Department of Interior 1996). This Record of Decision also established the Glen Canyon Dam Adaptive Management Program (GCDAMP) to carry out large scale experiments which would inform dam operations and other management options for conservation of key cultural, physical and biological resources (Department of Interior 1996). The GCDAMP is comprised of a diverse group of tribes, stakeholders, and management agencies which represent a variety of interests including conservation of resources in Grand Canyon National Park, recreational use of Colorado River resources, native fish conservation in the Colorado River ecosystem, and dam operations that meet water and power obligations of millions of people in the southwestern US (Gloss et al. 2005).

Throughout the Colorado River basin including Grand Canyon, native fish populations have declined in the decades following dam construction (Minckley et al. 2003). Humpback chub (Gila cypha), for example are now classified as “endangered” under the U.S. Endangered Species Act (ESA; Federal Register 32 FR 2001). In efforts to promote species recovery (U.S. Fish and Wildlife Service [USFWS] 1992) or to meet other regulatory requirements such as National Environmental Protection and Grand Canyon Protection Acts, the humpback chub has been a focal species in assessments of how ecosystem constituents respond to management actions (USDOI 1992; USDOI 1995). As such, the humpback chub is a species of key management interest and a proxy species for other ecosystem components in the Colorado River. The mechanisms for why humpback chub populations declined following dam construction remain unknown, but the leading hypotheses include (1) habitat changes related to flow modifications
and reductions in sediment input (Converse et al. 1998), (2) competition with or predation by non-native fish (Yard et al. 2011; Coggins et al. 2011; USDOI 2011), (3) non-native parasites (Hoffnagle et al. 2006), (4) or changes in water temperature (Clarkson and Childs 2000).

Since the inception of the Endangered Species Act of 1973 (ESA) the United States Fish and Wildlife Service (USFWS), in consultation with the Bureau of Reclamation, have issued a number of Biological Opinions on the operations of Glen Canyon Dam that identified warming the mainstem Colorado River as an essential action to create suitable habitat for humpback chub to spawn in the mainstem Colorado River (USDOI 1978, 1987, 1994, and 2008). Several laboratory and modeling studies have further suggested that warm water discharges from Glen Canyon dam may benefit native fish species through improvements in growth rate (Clarkson and Childs 2004; Petersen and Paukert 2005; Coggins and Pine 2010). This hypothesis has led to experimental low-flow releases (Trammell et al. 2002; Ralston 2011) to test these responses as well as consideration of a selective water withdrawal device on the upstream face of the dam to allow for water withdrawals above the thermocline (Vermeyen 2008).

Since about 2003, meteorological events including drought and low and high runoff levels (Pulwarty and Melis 2001; Wright et al. 2008) have combined to create unusual reservoir conditions. Water releases from the reservoir during this time are generally warmer than any in the preceding 30 years (Figure 1). While the annual water temperature variation is currently less than the pre-dam condition, the water temperatures in recent years are more similar to those expected under possible management actions (i.e., low summer steady flow, Trammel et al. 2002; Ralston 2011) or low reservoir levels related to climate change (Christensen et al. 2004).

In this paper, we compare growth of humpback chub during a period of cooler, more variable riverine conditions (about 1980-1993; Epoch 1) to growth during a period of warmer water and less variable flows (2001-2011; Epoch 2; Figure 2). Specifically we (1) characterize how the growth of humpback chub responds to warmer river temperatures and less variable discharge. Management actions that lead to improvements in humpback chub growth rates are of significant interest. Fish growth is an integration of a variety of biotic and abiotic factors (Devries and Frie 1996), and fish growth and survival are positively correlated (Lorenzen 2000;
Charnov et al. 2012). Both temperature and discharge have changed significantly in this river reach since the completion of Glen Canyon Dam (Figures 1 and 2). Temperature and discharge individually or in concert with other changes in the post-dam environment may have contributed to observed declines in humpback chub populations (Coggins et al. 2006a). Both factors could be actively managed through modifications to the dam intake structure or flow operations, but with significant costs in terms of construction, lost power revenue, and unmet water delivery obligations. (2) We improve on estimates and uncertainties of humpback chub growth across all ages for use in population assessment models (Coggins et al. 2006a, b; Coggins and Walters 2009), a need previously identified (Kitchell et al. 2003; Coggins et al. 2006a). These models are used to determine status and trends of this species in response to management actions for recovery planning. (3) We also offer alternative hypotheses to water temperature and flow variations that may explain observed growth patterns in this focal species. The information presented is useful for guiding reservoir operation and conservation planning actions for the Colorado River and other riverine ecosystems similarly impacted by changes in water temperature following dam construction.

Methods

Water temperature and flow during epochs – We downloaded water temperature records for Lee’s Ferry, Colorado River (USGS Gauge 0938000, USGS Grand Canyon Monitoring and Research Center data portal http://www.gcmrc.gov/dasa/default.aspx) as well as historical water temperature records from other USGS sources (S. Wright, USGS GCMRC) to construct a time series of annual water temperatures from 1950-2011. We visually compared seasonal temperature regimes before and after the dam was closed in 1963. We calculated temperature duration curves for Epoch 1 (1980-1993) and Epoch 2 (2001-2011) since the majority of fish in our samples were alive during these periods (see below). Our water temperature data were collected from the Lee’s Ferry gauge approximately 100 km upstream of the area where most of our fish samples were collected. While downstream warming does occur from the dam to the mainstem location where our fish were collected, we assumed that this warming rate and water temperature was mostly driven by reservoir elevation and hydrology. Therefore we did
not adjust the Lee’s Ferry temperature data. Thus our temperature estimates are conservative to what humpback chub in our study reach likely experienced (Wright et al. 2009).

Fish collection – Directed sampling of large numbers of humpback chub for otolith analyses was not possible due to their status under the US Endangered Species Act. Otolith samples from Epoch 1 were collected in 1989-1993 (n= 174) and were provided by Arizona Game and Fish sampling crews to D. Hendrickson (Texas Natural Science Center, University of Texas). Samples from Epoch 2, 2001-2011 (n=105), came primarily from incidental mortalities that occurred as part of standard fish monitoring activities by cooperating agencies or fish found dead from unknown causes (see Lindberg et al. in-review). Fish samples from both epochs came primarily from the Little Colorado River and downstream of its confluence with the mainstem Colorado River between river kilometers (rkm) 102-106 (as measured from Lee’s Ferry, AZ). This population of humpback chub is the largest in number of nine aggregations in this Colorado River reach between Lakes Powell and Mead (Valdez and Ryel 1997). The majority of sampled fish were captured using un-baited hoop nets (50-cm diameter x 100-cm length, 10-cm throat, 6-mm nylon mesh; as in Stone and Gorman 2006) fished for 24 hours. Within the mainstem Colorado River slow-speed boat electrofishing (pulsed DC current, 15-20 amps, 200-300 volts; boat speed 7-10 seconds per meter of shoreline) was used in addition to hoop nets during Epoch 2 (sampling restrictions prevented electrofishing in Epoch 1), particularly 2009-2011. Electrofishing is not possible in the Little Colorado River due to naturally high conductivity (>3000 µS).

Most humpback chub in this reach of the Colorado River undertake a potadromous spawning migration to the Little Colorado River (Gorman and Stone 1999). Although headwater reaches of the Little Colorado River are modified by agricultural and groundwater use, generally flows in the Little Colorado River are seasonally variable with peak flows coinciding with spring run-off and late summer monsoon rains. Water temperatures are also seasonally variable and, compared to the mainstem Colorado River, are warmer during spring, summer, and fall and cooler in winter.

Fish samples from both epochs were similar in size and collected in similar locations so we assumed they followed the same life history patterns and spent about the same amount of
time in the Little Colorado and mainstem Colorado rivers over the course of their lives. Our
growth estimates thus represent an integration of growth over the lifespan of the fish while
they use both habitats. Previous work (Gorman and Stone 1999; Coggins and Pine 2010)
suggests that humpback chub larger than 200-300-mm total length (TL) should spend most of
their time in the mainstem Colorado River although it is likely that some portion of the
population remain in the Little Colorado River throughout much or all of the year (Valdez and
Ryel 1995; C. Yackulic, USGS-GCMRC personal communication).

Otolith preparation and assessment – For fish collected from 1989-1993, detailed methods and
age estimates are available in Hendrickson (1993, 1997). Analytical methods used were
generally similar for the two Epochs. For Epoch 2, lapillar otoliths were dissected from the fish,
cleaned, dried, and embedded in epoxy (EpoFix or Epoxicure). Once embedded, frontal plane
sections were cut with an Isomet diamond saw (Buehler), polished down to 3 μm with
successively finer lapping paper (3M). Otoliths were photographed with transmitted light
microscopy (40-630X) for measurement and age determination using ImageJ (Ferreira and
Rasband 2012) to enhance growth bands and make measurements. Annual growth bands
(annuli) were counted on optical images or directly at the microscope. Similarly, daily
increments were counted from digital images and were often double-checked at the
microscope. Each otolith was read at least twice.

Ages were determined on as many fish as possible. Comparisons of ages were made by
the same reader on a sub-set of fish from both epochs and were found to be in close
agreement. We used daily ages of age-0 fish to calculate hatch-date distributions for humpback
chub in each Epoch (Figure 6). These daily ages were then converted to fractions of a year to
include in growth models with older fish. We accounted for differences in hatch date of older
fish (spring or fall) by assigning a birth date to all fish based on hatch-date distributions for that
specific epoch. This hatch-date was then calculated as a fraction of a year and added to the
annulus counts on each fish that daily rings were not enumerated on. Age at capture (in years
or fraction of a year) was plotted against total length (TL) at capture (converted from standard
length as necessary using equations from Hendrickson 1997 or derived from our own samples).

A von Bertalanffy growth model
\[ L_t = L_\infty (1 - e^{-k(t-t_0)}) \]  

was fit to these data where \( L_t \) = length at age \( t \), \( L_\infty \) = asymptotic length, \( k \) = metabolic rate, and \( t_0 \) is a constant of integration. A test of coincident curves was developed as a likelihood ratio test (Kimura 1980; Haddon 2001) to test for differences in the growth curves between epochs. For fish < age-3 we fit linear models of fish age and length using Analysis of Covariance (ANCOVA) to assess whether TL (response variable) differs as a function of age, epoch, and capture location (Colorado River or Little Colorado River) main effects.

**Results**

**Water flow and temperature**

We found discharge (flow, cubic-feet-per-second, CFS) to be higher and more variable during Epoch 1 than Epoch 2 (Epoch 1 annual mean = 16,093 CFS, 95% CI annual mean = 15,828-16,349 CFS; Epoch 2 annual mean = 12,347 CFS, 95% CI annual mean= 12,240-12,453 CFS; Figure 3). The highest annual flows were generally in the mid 1980’s and lowest annual flows were in the early 1990’s. A two sample t-test found significant differences in mean annual discharge between the two epochs (\( t = 1.95, df = 16.7, p\text{-value} = 0.03 \)). We found water temperatures in the mainstem Colorado River during Epoch 1 to be cooler and less variable than during Epoch 2 (Epoch 1 annual mean = 9.20 °C, 95% CI=9.12-9.24; Epoch 2 mean = 10.22°C, 95% CI=10.16-10.28; Figure 4). The coldest water temperatures were generally in the early 1990’s with warm temperatures and high variability observed in the early 2000’s. A two sample t-test found significant differences in mean annual water temperature between the two epochs (\( t = -2.56, df = 18.87, p\text{-value} = 0.02 \)).

Sample sizes, humpback chub size frequency distributions (Figure 5), and juvenile humpback chub hatch date distributions (Figure 6) of samples from both epochs were generally similar (Figure 5). The majority of samples from both epochs are small fish and less than 3 years of age (Figure 5). Visual assessment of von Bertalanffy growth curves suggests good fits for both epochs with few outliers (Figures 7 and 8). Separate growth curves were fit to fish collected from each epoch and a single curve to data from both epochs was also fit (Figure 8). Growth model parameter estimates and uncertainty values are reported in Table 1. Overall we found differences in growth models between the two Epochs across ages (coincident curve test,
p<0.05). An examination of parameter values for the two models shows that that maximum theoretical size ($L_\infty$) and metabolic rate $k$ was higher, though with overlapping 95% confidence intervals, during Epoch 1 than Epoch 2 (Table 1).

For smaller (< 200-mm TL), younger (< age-3) fish, growth was linear (Figure 9) and was assessed separately from older fish. ANCOVA results testing for differences in slope between all main effects (Age*Epoch*Site interaction) were not significant (p=0.31) but between Epochs (Age*Epoch interaction) slopes were moderately significant (p=0.07), and slopes were significantly different between capture location (Age*Site interaction, p < 0.001). For the main effects Age and Epoch were highly significant (p<0.001 each), suggesting that length did differ by age (Age main effect) and that the intercepts are different (Epoch main effect) but Site was only moderately significant (p=0.08), suggesting that being captured in either the Little Colorado or mainstem Colorado rivers did not have a large effect on growth. A simplified regression analysis of age vs. TL by Epoch also suggests different, but overlapping slopes and intercepts (Figure 9; Table 2). An assessment of parameter values for these regressions suggests higher growth rates (larger slopes) and lower intercepts during Epoch 1 than Epoch 2 (Tables 2 and 3).

Discussion

Our evidence suggests that humpback chub growth patterns were different between Epoch 1 (1980-1993) and Epoch 2 (2001-2011), when environmental conditions also differed in terms of water temperature and flow variation. The growth trajectory of a fish integrates information on a wide range of environmental and ecological factors from predation risk and prey availability (Werner and Gilliam 1984) to habitat conditions and long-term trends in environmental conditions (Quinn and Peterson 1996; Rosenfeld and Boss 2001).

Based on water temperature and previous research, humpback chub growth rates were expected to be higher during Epoch 2, a period of warmer, more stable flows (Clarkson and Childs 2004; Petersen and Paukert 2005; Coggins and Pine 2010). We found generally similar growth rates (slopes) for smaller, younger humpback chub (< age-3 and < 200-mm) between the cold (Epoch 1) and warm (Epoch 2) periods (Figure 10, regression line). We also observed that for smaller fish, fish from Epoch 2 are often larger for a given age than Epoch 1 (Figures 9
and 10). However, for older fish (ages 2-10) the Epoch 1 fish are generally larger for a given age (Figure 8). Growth for fish across all ages shows higher theoretical maximum size and metabolic rate during Epoch 1 than Epoch 2 (Table 1). This suggests that growth may be improved for younger fish during Epoch 2, but for older fish growth was faster during Epoch 1 - a counterintuitive result which suggests the influence of factors other than temperature. This result is important for informing hypotheses related to operations of Glen Canyon Dam and management actions in the Colorado River such as future flow experiments or other actions to alter temperature to minimize extinction risk and promote population recovery of humpback chub (USDOI 2002).

There are several possible reasons why younger fish grew faster during Epoch 2 while older fish grew faster during Epoch 1. First, water temperatures during Epoch 2 compared to other time periods in a post-Glen Canyon Dam river environment were only marginally warmer than the post-dam annual temperature range, particularly seasonal maximum temperatures. Modeling results have suggested that maximum growth improvements are predicted for changes in temperature of 10°C or more (water temperatures from 10°C to 20°C; Coggins and Pine 2010) substantially more than the 1.5-2°C average increase in temperature observed for more than 90% of the time in Epoch 2 compared to Epoch 1 (Figure 4).

Second, river flows were generally lower and less variable in Epoch 2 than Epoch 1, other than the infrequent controlled floods (Melis et al. 2012). Lower, more stable river flows may have altered invertebrate foodbase composition and production which could impact fish populations. Finch et al. (in-review) assessed how juvenile humpback chub growth responded to a 60-day steady flow experiment in the Colorado River (in comparison to the extant daily fluctuating flows) and found that growth rates during the steady flow periods were actually lower than growth rates during fluctuating flows. One potential mechanism for this response is changes in invertebrate drift concentrations during steady vs. fluctuating flows. Greater flow variation likely increases vulnerability of invertebrates in drift and available as prey to fish (T. Kennedy USGS-GCMRC, personal communication). Following the 2008 high flow experiment in Grand Canyon, annual invertebrate biomass and production in the Lee’s Ferry reach of the Colorado River below Glen Canyon Dam declined by more than 50% primarily due to declines in
New Zealand mudsnail *Potamopyrgus antipodarum* and amphipod *Gammarus lacustris*. Large increases in populations of drifting invertebrate species (Chironomidae and Simuliidae) and juvenile rainbow trout likely benefited from increases in drift for over a year following the flood (Cross et al. 2011; Korman et al. 2012).

As observed in many regulated river systems, particularly in the arid southwestern U.S., macroinvertebrates that were once found in Grand Canyon have been replaced by a less diverse taxa nonnative assemblage dominated primarily by chironomids and simuliids (Blinn and Cole 1991; Stevens et al. 1997; Oberlin et al. 1999; Vinson 2001). These invertebrate taxa continue to remain selectively depauperate, and small in size, likely due to ecosystem modifications post-dam of stable flow, lower turbidity, and less variable temperatures (Stanford and Ward 2001; Cross et al. 2013). Of the introduced taxa, larger sized macroinvertebrates like non-native amphipods (*Gammarus sp.*) are often highly selected by the native and non-native fish community. *Gammarus*, for example, have been found to comprise a high proportion of humpback chub diet when available (Valdez and Ryel 1995). While data on humpback chub diet through time is limited, there is some evidence that the occurrence of certain prey resources may have shifted over recent decades in the area around the Little Colorado River (Kaeding and Zimmerman 1983; Kubly 1990; Valdez and Ryle 1995; Valdez and Hoffnagle 1999; Cross et al. 2013) for unknown reasons (Pinney 1991; Blinn et al. 1995).

Possible reasons for the reduction in prey availability between Epoch 1 and Epoch 2 are (1) amphipods are now less vulnerable to drift entrainment because of reduced daily flow variability (Modified Low Flow Fluctuations, T. Kennedy, USGS-GCMRC personal communication), or (2) amphipod abundance is lower due to reduced algal production from scenscent growth and increased respiration due to warmer water temperatures during winter when light availability is reduced (Yard 2003). Although *Cladophora glomerata* is not an important food resource for humpback chub this green alga serves as a biological substrate for epiphytes like diatoms and as a refuge and food resource for invertebrates particularly *Gammarus* (Blinn et al. 1995; Shannon et al. 1994). Therefore, in this regulated river the availability of *Gammarus* as a prey item for chub is likely to be negatively correlated with reduced flow fluctuations and increased winter water temperatures.
Our analysis for adult fish could be improved if there were humpback chub age samples available in the approximately 250-350-mm size range (likely fish age-10 to age-30) from epoch 2. As a federally listed endangered species the directed take of adult humpback chub for age analyses has not been permitted since the Hendrickson samples were collected in 1989-1993. Our study made use of the Hendrickson (1993, 1997) samples as well as incidental mortalities from ongoing sampling efforts or fish simply found dead in the river (i.e., post-spawning mortality). Recently, we developed an individual-based population viability analysis model to assess the risk to humpback chub populations in this same area for large mortality events or directed take as part of research activities (Pine et al. 2013). Results from Pine et al. (2013) suggest that extinction risk from directed sampling of small numbers of humpback chub juveniles for age sampling is negligible and that the population is highly resilient to removals of larvae and juveniles for research purposes. A similar approach could be used to assess the risk to the population from directed take of adult fish in these “missing” size and age classes.

A close examination of the growth data that are available shows that the slopes of the fitted growth curves from the linear regression models and von Bertalanffy models both cross between age-2 and age-3 (Figures 8 and 10). At ages past this inflection point, fish across all ages in Epoch 2 appear to have lower growth rates and size at age (both observed and predicted), including ages 3-10 where sample sizes between the two epochs for size at age information are more comparable (Figure 8). These fish (ages 3-10) are perhaps of greatest interest in this growth comparison between the two epochs because their entire growth trajectory occurred under observed conditions. In contrast, the largest, oldest humpback chub from Epoch 2 would have lived at least the first third of their life during the conditions observed late in Epoch 1 so their early life would have been spent under conditions observed late in Epoch 1. We acknowledge that our results may be biased by lack of data in these missing cohorts and encourage future research efforts to fill this important data gap if possible.

Other factors beyond temperature and discharge could also alter growth rates. We developed a matrix of factors that could have influenced growth differences between these two epochs. These factors included additional physical parameters such as changes in flow volume or flood frequency in the Little Colorado River as well as a group of biological effects including
introduction of parasites in the Little Colorado River (Choudhury et al. 2004; Hoffnagle et al. 2006), differential survival of juveniles related to changes in predator abundance in the mainstem Colorado River (Korman et al. 2012; Coggins et al. 2011) or Little Colorado, or density dependent effects on growth from increases in humpback chub abundance. Coggins et al. (2006a) found large declines (30-60%) in abundance of humpback chub ≥ 150-mm TL in this aggregation of humpback chub beginning in the early 1990s that was followed by a period of low but stable population size in the early 2000s. Coggins and Walters (2009) identified a trend of increasing abundance of adult humpback chub beginning about 2007 based on reconstructed abundance trends. Van Haverbeke et al. (2013) found a significant increase in humpback chub and other native species including flannelmouth sucker *Catostomus latipinnis* and bluehead sucker *Catostomus discobolus* in the Little Colorado River since about 2006 for fish ≥ 150-mm TL and ≥ 200-mm TL from field-based mark-recapture and catch-per-unit-estimate (CPUE) estimates. Van Haverbeke et al. (2013) highlight that these increases in three native fish species suggest a systemwide change that benefited these species. However it is not possible to isolate other factors that may have led to this population increase considered by these authors and others (Coggins et al. 2011) including warmer mainstem water temperatures since 2003 and decreases in non-native species at the Little Colorado River-mainstem Colorado River confluence reach from 2003-2006 (Yard et al. 2011).

All of our fish from Epoch 2 < 200-mm TL were born in 2005 or later with the majority born in 2009-2010 during this time period of higher juvenile humpback chub abundance. Could these increases in abundance lead to density dependent growth effects, causing lower growth? Examination of the size at age and growth (slopes) of the age-length relationship (Figure 10) shows that size at age was generally larger for fish of a given age until sometime between age-2 to age-3 when the slopes intersect. This is primarily because of a larger y-intercept for fish from Epoch 2, but an examination of the data for fish ≤ 150-mm TL shows that in general the fish from Epoch 2 are larger for a given age than fish from epoch 1, not smaller as density dependent growth may suggest. Hayes et al. (*in-review*) also found that juvenile humpback chub in the Little Colorado River had higher body condition during this same time period of increasing humpback chub abundance than earlier years in the Little Colorado River.
further suggesting that density dependent effects are not manifest in terms of lower growth. Hayes et al. (in-review) also found that the smallest juvenile humpback chub in their study had similar body condition for a given size across a range of years up until about 120-mm TL. Above this, body condition was lower for fish in the Little Colorado River than mainstem Colorado River. This is about the same inflection point we observed where the slopes of the age-length curves cross between the two epochs. While it appears that density increases did not affect growth, it is unknown if density dependent effects may serve to trigger juvenile humpback chub to egress from the Little Colorado River to the mainstem Colorado River (Chapman 1966; Finch et al. in-review; Limburg et al. in-review).

Conclusions

We were interested in assessing humpback chub growth responses to two different epochs because growth is a metric that integrates a large suite of physical, biological, and ecological aspects of an animal’s environment than can be manipulated via management actions. Our results suggest that growth patterns of humpback chub may have changed in this river reach over the past 30 years, but, we are unable to identify a single causal mechanism for this change from the available data. Our findings suggest juvenile humpback chub growth are presently larger, and adults smaller, for a given age in this reach of the Colorado and Little Colorado rivers than observed in the 1980s and 1990s. This may be related to changes in key aspects of the physical and biological conditions in the Colorado River ecosystem observed in recent years including warmer water temperatures, lower predator densities, and higher abundances of congenerics. The differential response in growth between juvenile and adult humpback chub is interesting because juveniles would most likely have spent most of their life in the Little Colorado River which has not been altered to the same extent as the mainstem Colorado River. This suggests that conditions may have changed in the Little Colorado River independent of management actions in the mainstem Colorado River as well which support faster juvenile growth. This is an important area of future research. Foodbase resources important to juvenile and adult humpback chub may also have changed during this time period but the available information on is an area of limited information that could be addressed in future research. Given the highly dynamic nature of large arid climate rivers such as the
Colorado and Little Colorado rivers, it is likely that variations in growth simply reflect the evolutionary plasticity humpback chub have evolved and express while living in these two very different river systems. Future work should continue to fill missing data gaps (especially for age-10 to age-30 humpback chub) and compare growth patterns of humpback chub from this highly regulated reach of the Colorado River with those in a partially regulated yet less modified environment (Muth et al. 2000; Stanford and Ward. 2001).

Acknowledgements

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Table 1. Parameter estimates for VB curves.

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<th>Upper 95% C.I.</th>
<th>Study</th>
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<td>$k$</td>
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Table 2. Linear regression parameters for a TL~Age model for humpback chub < age-3 that were collected in either the Little Colorado River (LCR) or Colorado River (CO) during epoch 1 or epoch 2.

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<th>Slope</th>
<th>Slope SE</th>
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Table 3. Linear regression parameters for a TL~Age model for humpback chub < age-3 by Epoch.

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<th>Slope SE</th>
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References


*Available in this report*
Figure 1. Time series of water temperature measurements taken at Lee’s Ferry, Colorado River, Arizona using various methods and sources with most recent data collected at USGS Gauge 0938000. The vertical red line indicates when Glen Canyon Dam was completed in 1963. Breaks in the line are due to missing data for this gauge station.
Figure 2. Discharge (CFS) measured at Lee’s Ferry, Colorado River, Arizona from 1921-2011. Measurements taken at USGS gauge station 0938000. The vertical red line indicates the completion of Glen Canyon Dam in 1963.
Figure 3. Flow summary information measured at Lee’s Ferry, Colorado River, Arizona taken at USGS gauge station 0938000 for Epoch 1 (1980-1999) and Epoch 2 (2001-2011). The top left is the flow duration curve for each epoch, top right are box plots of annual flow for each epoch, bottom left are the mean annual flows for each epoch and the bottom right is the coefficient of variation (CV) in annual flows for each year.
Figure 4. Water temperature (degrees C) summary information measured at Lee’s Ferry, Colorado River, Arizona taken at USGS gauge station 0938000 for Epoch 1 (1980-1999) and Epoch 2 (2001-2011). The top left is the water temperature duration curve for each epoch, top right are box plots of annual water temperature for each epoch, bottom left are the mean annual water temperatures for each epoch and the bottom right is the coefficient of variation (CV) in annual water temperatures for each year. Breaks in the line are due to missing data for this gauge station.
Figure 5. Size (top panel, TL, mm) and age (bottom panel, years) density plots for humpback chub collected during Epoch 1 (blue dashed line, Hendrickson) and Epoch 2 (red solid line, NSE).
Figure 6. Monthly hatch-date distribution density plots for juvenile humpback chub collected during 1989-1993 (Epoch 1, blue dashed line) and 2001-2011 (Epoch 2, solid black line).
Figure 7. Von Bertalanffy growth curves (solid lines) and 95% confidence intervals (dashed lines) fit to age (x-axis) and size (TL, mm, y-axis) data for humpback chub in the Colorado River, Arizona. Data came from two different time periods, Epoch 1 (top left) were collected in 1989-1993 and Epoch 2 (top right) were collected in 2001-2011. A single von Bertalanffy growth curve was also fit to data from both epochs combined (bottom left).
Figure 8. Von Bertalanffy growth curves fit to humpback chub age (x-axis) and size (TL, mm, y-axis) from Epoch 1 (blue lines and circles) and Epoch 2 (red lines and triangles). Solid lines represent the fitted line (fit to data from each epoch separately) and the dashed lines represent the 95% confidence intervals for the predicted size for each age.
Figure 9. Linear regressions of age (x-axis, fractions of a year) and total length (y-axis, TL, mm) fit to humpback chub < age-3 by epoch (Epoch 1 top row, Epoch 2 bottom row) and by collection river (LCR = Little Colorado River, CO = Colorado River).
Figure 10. Linear regressions of age (x-axis, fractions of a year) and total length (y-axis, TL, mm) fit to humpback chub < age-3 by epoch (Epoch 1 solid black line and filled circles, Epoch 2 dashed line and open circles).
This is the end of the report. For corrections, updates, and reprints of manuscripts as they become available see:

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Thank you.